UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE CIENCIAS BIOLÓGICAS

TESIS DOCTORAL

Aplicación de métodos de secuenciación paralela masiva y genómica al estudio de variantes génicas que regulan: crecimiento, conformación y calidad de carne en cerdo

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Ángel Mario Martínez Montes

Directora

Ana Isabel Fernández Ávila

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Resumen

Las estrategias de análisis llevadas a cabo hasta ahora para la identificación de la base genética de caracteres complejos no han resultado del todo eficientes, en parte debido a la sobreestimación de los efectos y la aparición de falsos negativos y de falta de validación en fondos genéticos distintos. Es por ello, que la aparición de nuevas metodologías de análisis masivo del genoma, que se encuentran en pleno desarrollo, pueden aumentar la potencia de este tipo de análisis, proveyendo nueva información a precios relativamente asequibles que permiten superar estas limitaciones.

El presente trabajo de tesis doctoral se basa en la gran cantidad recursos, datos y resultados de detección de QTL, genes candidato y expresión génica diferencial llevados a cabo a partir de la población experimental Ibérico x Landrace (IBMAP) y posteriores generaciones. El objetivo del presente trabajo ha sido explorar diferentes aproximaciones basadas en el estudio masivo del genoma porcino para profundizar en el conocimiento de la base genética de caracteres productivos, específicamente los relacionados con crecimiento, deposición grasa y rendimiento de piezas nobles en tres retrocruces experimentales F1 (Ibérico x Landrace) x Landrace, F1 (Ibérico x Duroc) x Duroc y F1 (Ibérico x Pietrain) x Pietrain. Para abordar este objetivo se han planteado tres aproximaciones que han dado lugar a tres estudios.

El objetivo del primer estudio fue la identificación de SNPs mediante secuenciación del ARN de hígado e hipotálamo, RNA-Seq, en el retrocruce Ibérico x Landrace. A partir un diseño experimental basado en la comparación de grupos extremos para crecimiento y deposición grasa, se pudieron identificar más de 90.000 SNPs, de los cuales 4.396 SNPs identificados en hipotálamo y 1.862 en hígado, estarían potencialmente relacionados con la variabilidad de los caracteres, proporcionando una relevante base de datos de mutaciones candidatas. Además, se identificaron fenómenos de edición de ARN, mostrando que este no es un fenómeno despreciable y que puede ser responsable de la variabilidad de caracteres de interés. Además de afectar la tasa de falsos negativos al validar polimorfismos.

El objetivo del segundo estudio fue descifrar cómo funciona la regulación de la expresión de genes relacionados con los caracteres productivos mediante genética genómica. Para ello, se hizo un análisis de asociación de genomas completos (GWAS), utilizando información de genotipado masivo y datos preseleccionados de expresión génica de músculo *Longissimus dorsi* en los animales del retrocruce Ibérico x Landrace. Los resultados obtenidos han permitido poner de manifiesto la complejidad de la

regulación génica, identificando más de 60 eQTL, de los cuales seis solapan con QTL identificados en estudios previos en el mismo material. A partir de estos resultados y enfocado a los QTL solapantes se identificaron genes y mutaciones candidatas relevantes, explotando la información de identificación de SNPs del primer trabajo. Entre los genes propuestos destaca *ALDBSSCG0000001928* que corresponde a un ARN largo no codificante (ARNlnc), elementos clave en la regulación génica.

El objetivo del último de los estudios fue validar regiones QTL para caracteres productivos en tres fondos genéticos distintos basados en cerdo Ibérico, retrocruces Ibérico x Landrace, Ibérico x Duroc e Ibérico x Pietrain. Para ello, se realizaron análisis de asociación de genomas completos en cada uno de los retrocruces y en el conjunto del material. Estos análisis han permitido identificar 22 regiones QTL comunes, incrementando probablemente el éxito en la identificación de QTNs. Pero, además, los resultados ponen de manifiesto la relevancia de los QTL retrocruce-específicos, 58, que muestran efectos concordantes con los fondos genéticos analizados. Además, a partir de estos resultados e integrando resultados de expresión génica diferencial y de identificación de SNPs del primer trabajo, se identificaron genes y mutaciones candidatas relevantes. Además, la combinación de este estudio con el anterior, permite destacar 18 regiones QTL asociadas a caracteres relacionados con deposición grasa y rendimiento de piezas nobles y a diferencias de expresión génica, identificadas de manera independiente en cada una de las aproximaciones, potenciando su valor.

Finalmente resaltar que los estudios realizados bajo esta tesis doctoral han generado gran cantidad de información y resultados que serán útiles en estudios futuros sobre este y otro material animal, además de justificar el planteamiento de estudios más específicos enfocados al análisis de los genes y mutaciones candidatas propuestas.

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Summary

The analysis strategies carried out for the identification of the genetic basis of complex traits have not been completely efficient so far, in part due to the overestimation of the effects and the occurrence of false negatives and lack of validation in different genetic backgrounds. For this reason, the emergence of new methodologies of massive analysis of the genome, which are in full development, can increase the power of this type of analysis, providing new information at relatively affordable prices that overcome these limitations.

The current thesis is based on the great amount of resources, data and results of detection of QTL, candidate genes and differential gene expression studies carried out in the experimental population Iberian x Landrace (IBMAP) and later generations. The objective of the present thesis was to explore different approaches based on massive analysis of the porcine genome to deepen the knowledge of the genetic basis of productive traits, specifically those related to growth, fat deposition and premier cut yields in three experimental backcross F1 (Iberian x Landrace) x Landrace, F1 (Iberian x Duroc) x Duroc and F1 (Iberian x Pietrain) x Pietrain. Three approaches have been proposed to address this objective and have led to three studies.

The objective of the first study was the identification of SNPs from RNA sequencing, RNA-Seq, data from liver and hypothalamus of Iberian x Landrace backcrossed pigs. The experimental design based on the comparison of extreme groups for growth and fat deposition, allowed us to identify more than 90,000 SNPs, 4,396 SNPs identified in the hypothalamus and 1,862 in the liver that would be potentially related to the trait variability, providing a relevant database of candidate mutations. In addition, RNA editing phenomena were identified, showing that this is not a negligible phenomenon and may be responsible of trait variability. In addition, it could be affecting the false negative rate when validating polymorphisms.

The aim of the second study was to decipher how the regulation of gene expression associated with productive traits works using a genomic genetics approach. For this, a genome wide association analysis (GWAS) was carried out, using high density genotyping data and pre-selected gene expression data of *Longisimus dorsi* muscle from the Iberian x Landrace backcrossed pigs. The results obtained showed the complexity of the gene regulation, identifying more than 60 eQTL, six of them overlapping with QTL for phenotypic traits identified in previous studies in the same material. Moreover, the

examination of the overlapping QTL allowed us to identify relevant candidate genes and mutations, exploiting the SNP information generated in the first work. Among the proposed genes highlights *ALDBSSCG0000001928* that corresponds to a long non-coding RNA (ARNlnc), key elements in gene regulation.

The objective of the last study was to validate QTL regions for productive traits in three different genetic backgrounds based on Iberian pig, Iberian x Landrace, Iberian x Duroc and Iberian x Pietrain backcrosses. To achieve this objective, backcross independent and joint genome wide association analyses were conducted. These analyses have allowed us to identify 22 common QTL regions, probably increasing the success in the identification of QTNs. But, the results also revealed the relevance of backcross-specific QTLs, 58, which showed concordant effects with the analysed genetic background. Additionally, by integrating results of differential gene expression studies and identification of SNPs from the first study, relevant candidate genes and mutations were identified. Moreover, the combination of these results with the previous ones, allowed us to highlight 18 QTL regions associated with fat deposition and premier cut yield related traits and gene expression differences, supporting their value.

Finally, note that the studies carried out under this doctoral thesis have generated a large amount of information and results that will be useful in future studies on this and other animal material. In addition, these results lead to promote proposals for more specific studies focused on the analysis of the proposed candidate genes and mutations.

Introducción

1 - Visión global de la evolución de la mejora en especies ganaderas

Los programas de mejora animal se basan en la identificación y selección de caracteres de interés, caracteres que varían en función de la especie animal, el lugar y el momento en el que se desarrollan dichos programas. Tradicionalmente este proceso se ha llevado a cabo mediante la selección a partir de la información disponible de los propios candidatos o selección masal e incluyendo la información del pedigree en el proceso. Posteriormente, esta selección se basó en estimas del valor mejorante de los animales mediante la metodología BLUP (Henderson 1984; Gianola *et al*. 1986). Aunque estos métodos han llevado a obtener una gran mejoría en diferentes caracteres tanto productivos como reproductivos y funcionales, existen ciertas limitaciones debidas en parte al esfuerzo requerido para la obtención de datos fenotípicos, la correlación positiva o negativa existente entre ellos, así como por la necesidad de medir la mayoría de los caracteres en los individuos adultos, alargando así el periodo entre generaciones y aumentando por ello el tiempo y el coste de cría. Es por ello que la incorporación de nuevas herramientas genéticas y genómicas, que permiten estudiar la base genética de estos caracteres, en los programas de mejora puede aportar grandes beneficios (Van Eenennaam *et al*. 2014).

Uno de las primeras aproximaciones al estudio de la base genética de caracteres de interés en especies ganaderas fue empleando como marcadores los grupos sanguíneos, para mapear loci para caracteres cuantitativos (*Quantitative trait loci*, QTL) en vacuno (Rendel 1961). Posteriormente se han llevado a cabo gran cantidad de estudios basados en el análisis de genes específicos, principalmente para caracteres mendelianos, en distintas especies ganaderas. Un claro ejemplo es el gen del *halotano* en porcino (Fujii *et al*. 1991).

Sin embargo, para caracteres poligénicos existe una clara dificultad en la identificación de su base genética. Con este objetivo se han diseñado diferentes estrategias de análisis basadas en la detección de QTL con marcadores genéticos anónimos, principalmente mediante el genotipado de microsatélites y actualmente polimorfismos de un solo nucleótido (SNPs) y en estudios de genes candidatos específicos. Sin embargo, estas estrategias de análisis no han resultado del todo eficientes, en parte debido a la sobreestimación de los efectos y la aparición de falsos negativos cuando los efectos detectados son de pequeña magnitud (Beavis 1998). Además, en la mayoría de los casos los efectos detectados en un material animal especifico no suelen ser validados en otros

materiales y fondos genéticos distintos (Van Eenennaam *et al*. 2014). Es por ello que la aparición de tecnologías y metodologías de análisis masivo del genoma, que se encuentran en pleno desarrollo en la actualidad, puede aumentar la potencia de este tipo de análisis, proveyendo nueva información genómica a precios relativamente asequibles que permiten superar estas limitaciones. Dentro de estas nuevas metodologías, una de las más valoradas es la selección genómica (Goddard and Hayes 2007), descrita en origen como una estrategia de predicción de los valores mejorantes a nivel de genoma mediante el uso de paneles densos de SNPs (Meuwissen *et al*. 2001).

1.1 Estado actual de la mejora en porcino

El sector porcino es uno de los sectores más relevantes en la producción agraria en España, siendo el principal sector económico a nivel de producción final ganadera, representando el 37% de la producción total, que llega a alcanzar casi los 6.000 millones de euros al año. Aunque la tendencia hasta 2013 ha sido a la disminución en el número de granjas, en estos últimos años ha sido revertida pasando de 85.449 en 2013 a 87.553 en 2015, viéndose aumentado además el consumo de carne alrededor de un 3% frente al año 2014 (Magrama 2015).

Tabla 1: Tabla resumen de los atributos de la carne en función del tipo de calidad al que afectan, considerando la calidad sensorial (percepción por el consumidor), calidad nutritiva (relacionada con la composición en grasa y colesterol) y calidad tecnológica.

Categoría	Atributos
Calidad sensorial	Color
	Terneza
	Jugosidad
	Sabor
	Olor
	Cantidad de grasa visible
	Veteado
Calidad nutritiva	Cantidad de grasa
	Composición de ácidos grasos
	Valor proteico
Calidad tecnológica	pH
	Capacidad de retención de agua
	Consistencia de la grasa
	Separación de tejidos
	Estabilidad oxidativa

Actualmente, el principal objetivo de la mejora porcina se centra en la calidad de la carne, que se define como el conjunto de todas las características que afectan al valor del producto final, tanto tecnológicas, como nutricionales y sensoriales (Tabla 1).

La calidad sensorial u organoléptica depende de diferentes factores como la relación entre músculo y grasa o la composición muscular, siendo de gran relevancia los depósitos lipídicos y la composición específica de ácidos grasos, afectando directamente a la calidad de la carne. El depósito graso más relevante en la calidad de carne corresponde a la grasa intramuscular (GIM) que se localiza entre las fibras musculares, favoreciendo la terneza, el sabor y la jugosidad de la carne (Le Dividich *et al*. 1991). Un ejemplo que pone de manifiesto la importancia de estos caracteres, así como la dificultad de la selección aplicada a éstos, es el intento para mejorar el rendimiento magro de la canal mediante la selección en contra del espesor de grasa dorsal (GD). Sin embargo, debido a que existe correlación genética positiva entre GD y GIM, esta tendencia en la selección ha provocado una reducción de la GIM y su consecuente reducción de calidad de la carne (Toro and Silió, 1992, Solanes *et al*. 2009), haciéndose necesario el desarrollo de métodos que permitan modificar la cantidad de GD y GIM de manera independiente. También hay que tener en cuenta la calidad nutricional del producto, afectando a caracteres tales como la composición de ácidos grasos y triglicéridos, cantidad de grasa y valor proteico, que pueden tener un efecto directo sobre la salud humana.

Además, hay que remarcar los aspectos relacionados con el bienestar animal, que están siendo cada vez más relevantes para la sociedad, hasta el punto de haberse desarrollado una regulación completa al respecto, tratando diferentes aspectos como la protección de los animales tanto en la granja, como durante el transporte e incluso en el proceso de sacrificio (Phocas *et al*. 2016). Por último, los caracteres que afectan a la salud del animal son también relevantes en los programas de mejora, ya que la selección hacia animales más robustos, con una mayor resistencia general a enfermedades, afecta directamente a los valores finales de producción y rendimiento (Van Eenennaam *et al*. 2014).

Entre las razas porcinas más usadas en la industria se encuentran las siguientes: Landrace, que presenta buena aptitud materna, buena ganancia media diaria y bajo nivel de engrasamiento, así como buen rendimiento a la canal; Duroc, caracterizada por su alta calidad de la carne, debido al nivel de grasa infiltrada, además de presentar alta prolificidad, Pietrain, que es la raza con mayor porcentaje de músculo, pero con bajos

parámetros de crecimiento y prolificidad. En la producción de carne y productos derivados de alta calidad en la industria porcina española destaca la raza Ibérica, que es la única raza española bajo producción industrial, característica por su alto contenido en grasa intramuscular y perfiles particulares de composición de ácidos grasos, especialmente por su alto contenido en ácido oleico. Se trata de animales con tendencia a acumular depósitos grasos que se infiltran en la masa muscular (German *et al*. 2005). Debido a estas características, se considera que esta carne es de alta calidad y sus productos curados presentan excelentes propiedades sensoriales (López-Bote 1998).

El sistema actual de producción de cerdo ibérico se basa en dos tipos genéticos diferentes, raza pura Ibérica y cruces Duroc x Ibérico. En los últimos años predomina el cruce Duroc x Ibérico, ya que muestra ciertas ventajas frente a la raza pura a nivel de conversión alimenticia y el rendimiento de piezas nobles (Serrano *et al*. 2009). Sin embargo, el uso de este material afecta a las propiedades organolépticas de manera negativa (Juarez *et al*. 2008). Actualmente, los esfuerzos en la mejora de este tipo de producción se centran en la identificación de las bases genéticas que regulan crecimiento y rendimiento, sin afectar a la calidad final tanto a nivel organoléptico y nutricional como a nivel de salud humana y bienestar animal. Es por ello que la incorporación de información molecular es considerada como una potente herramienta en la mejora de estos caracteres, debido a su baja heredabilidad, altas correlaciones y a la dificultad en la toma de sus registros (Miar *et al*. 2014).

Además de la importancia del sector porcino en la producción de carne, el cerdo se presenta como un importante modelo biomédico para el estudio de una gran variedad de enfermedades metabólicas, cardiopatías, diabetes, etc... Esto se debe a la gran similitud existente en el metabolismo y las características fisiológicas y anatómicas entre humanos y cerdos, convirtiéndolo en un modelo animal más adecuado frente a otros más usados tradicionalmente, como roedores (Spurlok and Gabler 2009).

2 - Análisis masivos de genomas

Una de las incorporaciones más relevantes al campo de la genética ha sido la aparición de tecnologías de análisis masivo del genoma, que permiten el desarrollo de diferentes estrategias de análisis en función de las necesidades y los medios disponibles.

2.1 Plataformas de genotipado masivo

Durante los 90 se emplearon principalmente marcadores microsatélite para todo tipo de estudios genéticos (Ollivier 2009). Sin embargo, el limitado número de microsatélites disponibles ha favorecido la incorporación y posteriormente su total remplazamiento por polimorfismos de nucleótido único (SNPs), que aun siendo menos informativos que los microsatélites, proporcionan una clara ventaja debido a su abundancia, automatización de genotipado e interpretación y coste. De hecho, se han puesto a punto múltiples plataformas para el genotipado de SNPs (Dekkers 2004) que incluyen desde pocas decenas hasta miles de SNPs (Fan *et al*. 2010) (Figura 1).

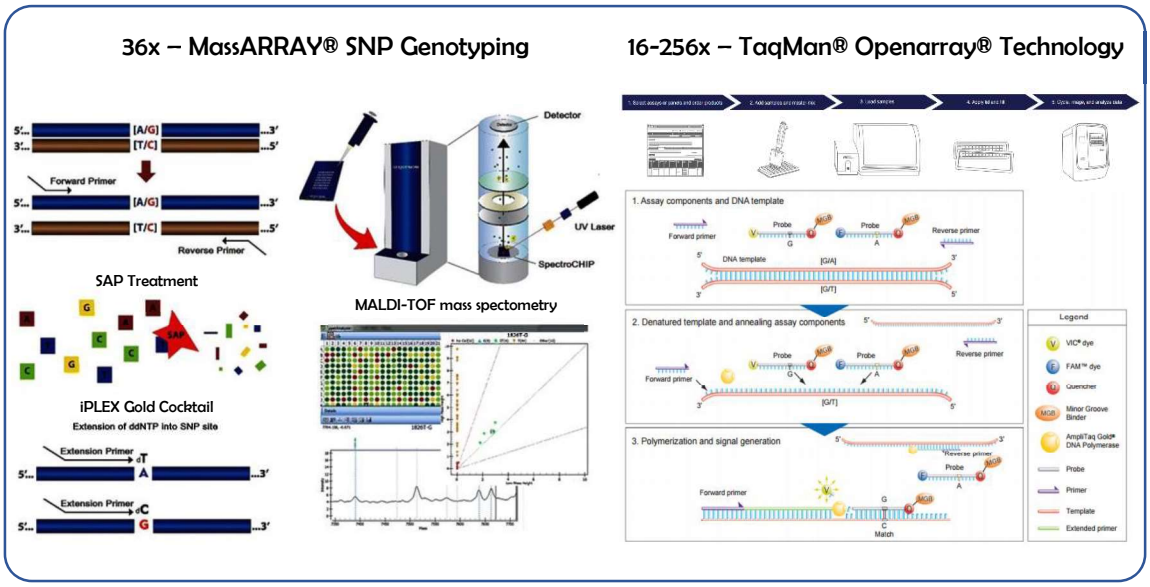

Figura 1: Ejemplos de tecnologías de genotipado de polimorfismos de baja y mediana capacidad

La formación del consorcio para la secuenciación del genoma porcino (SGSC) en 2003 permitió obtener la primera versión y anotación del genoma completo del cerdo en 2005 (Schook *et al*. 2005). Esta primera secuencia fue obtenida de un animal de la raza Duroc, con una cobertura media de 4x, y utilizando la tecnología de secuenciación denominada "*shotgun*", basada en la secuenciación clásica de fragmentos pequeños del genoma clonados en cromosomas bacterianos artificiales (BACs). Este primer ensamblaje del genoma dio pie a la identificación de un gran número de SNPs, que permitieron el desarrolló del chip comercial de genotipado masivo, PorcineSNP60 BeadChip (Illumina, Inc.) Ramos *et al*. (2009). Para el diseño de este chip se utilizaron las razas más comunes en producción (Landrace, Pietrain, Duroc y Large White) así como jabalí, incluyendo al

final un total de 64.232 SNPs, convirtiéndose en una herramienta de gran utilidad para muchos estudios que se han venido llevando a cabo en esta última década y aportando soluciones para algunas de las limitaciones existentes hasta el momento (Lillehammer *et al*. 2011; Tribout *et al*. 2012; Van Eenennaam *et al*. 2014).

A partir de la primera versión del genoma porcino y gracias a la información aportada por la comunidad científica, la secuencia del genoma porcino se ha ido ampliando y refinando, hasta llegar a la actual versión *Sscrofa10.2* (Groenen *et al*. 2012). A partir de toda la nueva información que se ha ido generando en estos últimos años sobre la secuencia de referencia porcina, en 2015 apareció una nueva plataforma de genotipado masivo, Axiom® Porcine Genotyping Array (Affymetrix, Inc.), con una mayor densidad de SNPs, desarrollado por Groenen (2015). En este caso la información usada correspondió a secuencias genómicas de las razas europeas más populares en producción (Landrace, Large White, Pietrain, Duroc y Hampshire) y razas locales, tanto europeas (14 razas de Reino Unido, Suecia, España, Italia) como asiáticas (8 razas de China y 1 de Tailandia), además de secuencias genómicas de jabalí tanto europeo como asiático. Para la selección de los SNPs incluidos en este chip, se seleccionaron aquellos que fueran bialélicos, mostraran frecuencias intermedias en los animales analizados y estuviesen espaciados de manera homogénea en el genoma. Además, se incluyeron alrededor de 56.000 SNPs de los incluidos en el chip previo de 60K, facilitando así la compatibilidad entre plataformas.

Tabla 2: Principales plataformas de genotipado masivo de SNPs en porcino. El coste incluye los reactivos necesarios y el genotipado en un servicio público

Plataforma	Nº SNPs	Año	Coste €/muestra
PorcineSNP60 v2 BeadChip	64.232	2012	108
Axiom® Porcine Genotyping Array	658.692	2015	142
GGP Porcine BeadChip	70.000	2016	55

Aunque la utilización de estas plataformas de genotipado masivo proporcionan mejoras evidentes, existe una tendencia enfocada al desarrollo y empleo de paneles de baja densidad específicos para caracteres, razas o poblaciones que junto con la imputación permiten utilizar dichos paneles para selección de forma económica y sencilla (Habier *et*

al. 2009; Wellmann *et al*. 2013). De hecho, ha surgido recientemente, un nuevo chip de genotipado de SNPs para porcino, desarrollado por GGP GeneSeek, que contiene 70.000 SNPs, 42.000 SNPs compartidos con el de 60K a un coste mucho más reducido (Tabla 2).

2.2 Secuenciación masiva

La aparición de la secuenciación masiva ha supuesto un gran avance en el estudio del genoma, ya que, en comparación con las técnicas clásicas, principalmente Sanger, basadas en la secuenciación individual de pequeños fragmentos de ADN amplificados, estas nuevas estrategias permiten la secuenciación de gran cantidad de fragmentos de ADN de forma paralela, disminuyendo en gran medida los costes y el tiempo necesario para secuenciar regiones genómicas de gran tamaño. Además, con la evolución de estas tecnologías, están apareciendo nuevas plataformas capaces de obtener secuencias de mayor tamaño, facilitándose así el proceso de secuenciación de genomas completos. Como se ha mencionado, el fundamento de estas técnicas es la secuenciación masiva paralela de fragmentos de ADN. Dichos fragmentos secuenciados, generados mediante diferentes técnicas, son posteriormente ensamblados hasta obtener *scaffolds* que unidos permiten determinar la secuencia completa de cada cromosoma y genoma (Figura 2).

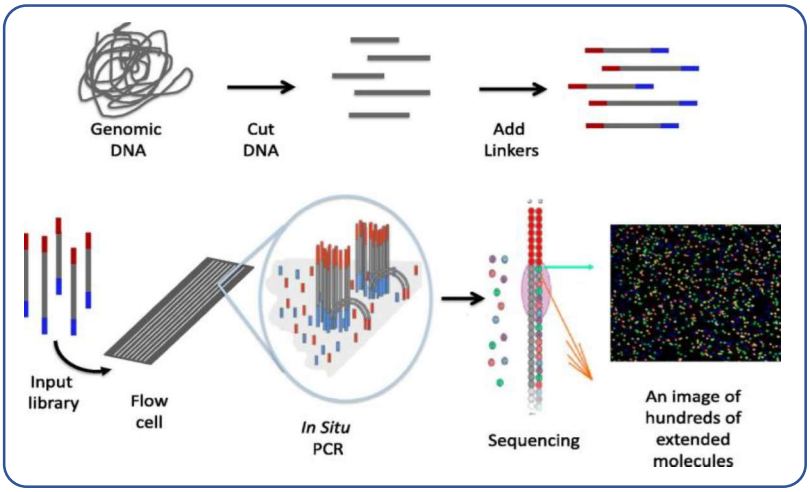

Figura 2: Esquema del proceso de secuenciación masiva (Johnsen *et al*. 2013)

Existen diferentes tecnologías de secuenciación masiva, en función del objetivo de estudio y medios disponibles, caracterizadas principalmente por la longitud de los fragmentos secuenciados. Estos pueden variar desde fragmentos pequeños de 85 pb con

la técnica de secuenciación por ligación (SoLiD) (Life Technologies), intermedios, entre 50-700 pb, como la basada en pirosecuenciación (Roche-454) o secuenciación por síntesis (Illumina), hasta fragmentos de gran tamaño entre 10-40 kb con las recientemente desarrolladas metodologías de secuenciación en tiempo real de molécula única (PacBio) e incluso fragmentos de hasta 500 kb que ofrece la secuenciación Nanopore (Oxford Nanopore). Estas tecnologías tienen propiedades diferentes que se adaptan a los requisitos de los usuarios, variando la precisión, el tiempo y los costes de secuenciación, ofreciendo una gran variedad de opciones a la hora de seleccionar la plataforma de secuenciación óptima para cada tipo de estudio.

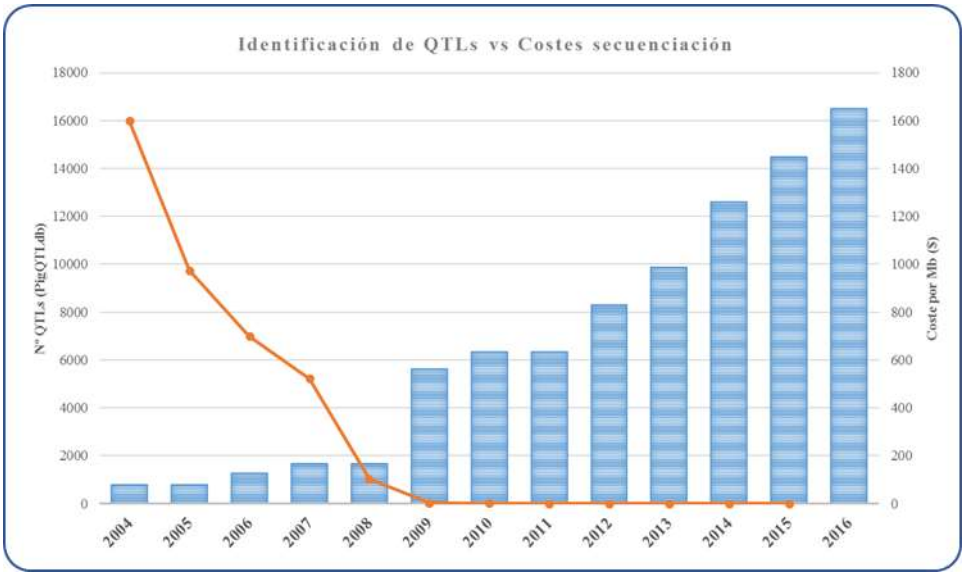

Figura 3: Evolución en el número de QTL identificados frente a los costes de secuenciación (--) y al número de SNPs disponibles () en porcino

La secuenciación de genomas completos es una de las principales dianas de la secuenciación masiva tanto en humano, como en especies modelos animales, así como en especies ganaderas. Se basa en la obtención de la secuencia completa de un organismo ya sea *de novo* o mediante mapeo frente a un genoma de referencia (resecuenciación), que ha favorecido el avance del conocimiento del genoma y su regulación. Claros ejemplos de éstos son el aumento en el número de variantes génicas identificados, la identificación de nuevos QTL y la implementación de técnicas de análisis ómicos basadas en secuenciación (ej. RNA-Seq). Esto ha sucedido gracias a la rápida evolución de las tecnologías de secuenciación masiva y la disminución de sus costes (Figura 3). Además,

ha abierto multitud de nuevas vías de estudio sobre el transcriptoma, el epigenoma y el metagenoma.

Secuenciación masiva y el genoma: La rápida evolución de las técnicas de secuenciación ha permitido la secuenciación del genoma de multitud de especies, desde humanos, plantas y animales, incluyendo especies relevantes por su función como especies modelo, así como en especies ganaderas y domésticas. En la actualidad podemos encontrar información sobre el ensamblaje y anotación de diferentes genomas completos en la base de datos de Ensmbl (www.ensembl.com). Sin embargo, la secuenciación *de novo* de genomas no es la única aplicación, sino que la resecuenciación de genomas permite la identificación de nuevos genes, pseudogenes, regiones reguladoras, etc… no descritos hasta el momento, así como el estudio de mutaciones genómicas tanto puntuales (SNPs, indels) como estructurales tipo *Copy Number Variants* (CNVs) (Feuk *et al*. 2006).

Estado actual del genoma porcino

La última versión de ensamblado y anotación del genoma porcino (Figura 4) corresponde la versión Sscrofa*10.2*, actualizado por última vez en febrero de 2014 (a 1 de abril de 2017). En esta última anotación se describe la existencia de 21.630 genes codificantes para 30.585 transcritos, 3.124 genes no codificantes (2.804 sncRNA y 135 ARNlnc) y 568 pseudogenes. Además, se describen más de 60.300.000 variantes cortas (SNPs e indels) y más de 800 variantes estructurales.

Summary

Assembly	Sscrofa10.2, INSDC Assembly GCA_000003025.4, Aug 2011
Database version	88.102
Base Pairs	3,024,658,544
Golden Path Length	2,808,525,991
Genebuild by	Ensembl
Genebuild method	Full genebuild
Genebuild started	Sep 2011
Genebuild released	May 2012
Genebuild last updated/patched	Feb 2014

Gene counts

Coding genes	21,630 (incl 10 readthrough)
Non coding genes	3,124
Small non coding genes	2,804
Long non coding genes	135 (incl 1 readthrough)
Misc non coding genes	185
Pseudogenes	568
Gene transcripts	30,585

Other

Genscan gene predictions	52,372
Short Variants	60,389,665
Structural variants	822

Figura 4: Sumario del estado actual de ensamblado y anotación del genoma porcino *S.Scrofa10.2* según Ensembl

Secuenciación masiva y el transcriptoma: Los estudios tradicionales del transcriptoma se centraban en el análisis de los niveles de expresión génica de un número determinado de tránscritos conocidos. Uno de los sistemas más utilizado con este propósito ha sido el de los microarrays de expresión, basado en el diseño de una colección de sondas de cDNA ancladas a una superficie sólida que se hibrida con el ARN de una muestra, para identificar los genes que están expresándose. Como ocurre con todos los estudios basados en la secuencia de ADN, la evolución en el conocimiento del genoma ha permitido el desarrollo de plataformas cada vez más complejas e informativas. En porcino los dos chips comerciales más utilizados son el *Affymetrix Porcine GeneChipTM* (Affymetrix), que contiene 23.937 conjuntos de sondas y el *Agilent Porcine Gene Expression Microarray* (Agilent) que contiene 43.803 sondas. Sin embargo, el desarrollo de las tecnologías de secuenciación masiva ha abierto nuevas vías en el análisis del transcriptoma, como es el caso del RNA-Seq, que permite estudiar el transcriptoma completo (Wang *et al*. 2009), de manera más eficiente que las tecnologías previamente usadas (Marioni *et al*. 2008). El procedimiento habitual de un análisis de RNA-Seq se basa en la fragmentación del ARN, síntesis de cDNA y su secuenciación mediante alguna de las tecnologías previamente descritas (Martin and Wang 2011) (Figura 5). Con esta tecnología, el análisis de los niveles de expresión génica se basa en el empleo de los valores normalizados del número de lecturas mapeadas contra cada transcrito, gen o exón. Una de las grandes ventajas que presenta la utilización del RNA-Seq frente a los microarrays es la capacidad de identificar todos los transcritos expresados, incluyendo nuevos genes o isoformas. En los últimos años esta tecnología ha permitido identificar múltiples genes candidatos para la regulación de caracteres productivos de interés, así como aproximaciones más complejas de redes génicas y biología de sistemas (en porcino: Ramayo-Caldas *et al*. 2012; Pérez-Montarelo *et al*. 2014; Wang *et al*. 2015; Zhang *et al*. 2015).

Además, esta técnica no está restringida a la búsqueda de transcritos y al análisis de los niveles de expresión, sino que al tratarse de una técnica basada en secuenciación permite analizar variaciones en la secuencia del ADN. Estudios previos han confirmado su utilidad como herramienta para la identificación y análisis de polimorfismos presentes en genes expresados (Chepelev *et al*. 2009; Cirulli *et al*. 2010). Sin embargo, hasta el momento el uso de RNA-Seq para la identificación de genes y mutaciones candidatos no ha sido aplicado de forma masiva en especies ganaderas (Koltes *et al*. 2015), siendo pocos

los estudios que se han llevado a cabo en este campo (Cánovas *et al*. 2010; Sharma *et al*. 2012). Además, la posibilidad de identificar alelos específicos en los transcritos que se están expresando permite la identificación de fenómenos de expresión alélica diferencial, así como de modificaciones post-transcripcionales como edición de ARN (Frésard *et al*. 2015).

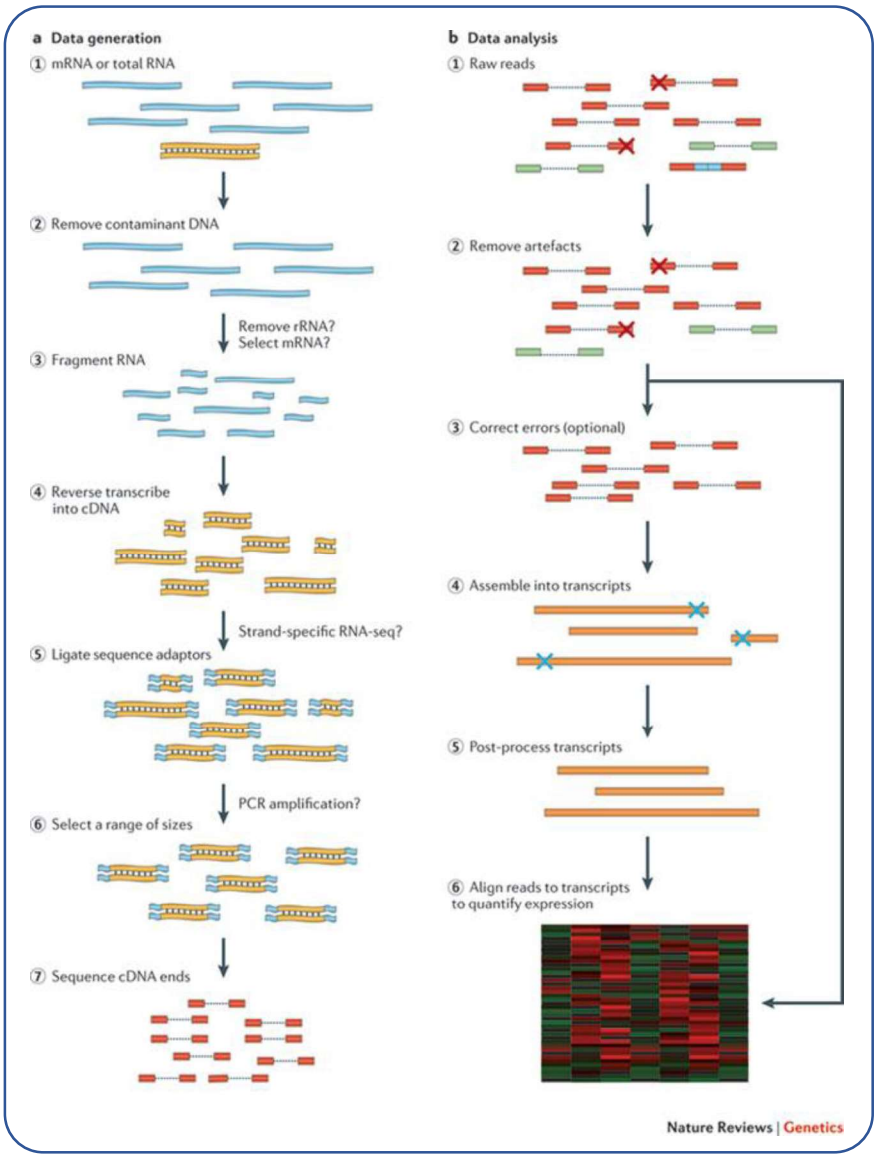

Figura 5: Pasos para la generación y análisis en un experimento típico de RNA-Seq (Martin and Wang 2011)

Secuenciación masiva y el epigenoma: El término epigenética fue definido por primera vez en 1942 como las interacciones causales entre los genes y sus productos generando un fenotipo determinado. El primer fenómeno epigenético estudiado se identificó al observar la heredabilidad de un carácter adquirido, obtenido a través de

tratamientos por estrés de calor, en la conformación de las alas en *Drosophila melanogaster* (Waddingtong 1942), fenómeno que se denominó como "asimilación genética" (Waddingtong 1953). Sin embargo, en aquella época se conocía poco sobre genómica y los posibles procedimientos que regulan este tipo de efectos. Con el paso de los años el campo de la epigenética ha evolucionado, en gran medida debido al aumento de conocimientos en biología molecular, regulación génica, y sobre todo con la aparición de las técnicas de secuenciación masivas. En la actualidad la epigenética incluye cualquier modificación que afecte a la expresión génica debido a factores diferentes a la mutación de ADN. Los casos más estudiados son; *Metilación de ADN:* basada en la metilación de citosinas en los dinucleótidos CpG, mediado por las metiltransferasas de ADN, y cuyo efecto varía en función de la región de metilación en la que ocurra. Este proceso afecta a la expresión de genes específicos, suprimiendo su actividad, además de intervenir en otros procesos como la inactivación del cromosoma X o la impronta genética; *Modificaciones post-transcripcionales de histonas:* basadas en modificaciones de acetilación, fosforilación, metilación y ubiquitinización, que afectan a la expresión génica debido a la variación de los niveles de condensación del ADN o regulando la interacción específica de proteínas con la molécula de ADN (Triantaphyllopoulos *et al*. 2016); *ARN no codificante (ARNnc):* se basa en la regulación de la expresión génica y la remodelación de la estructura del ADN mediada por ARNnc. Se han identificado ARNs no codificantes largos (ARNlnc) como el gen *XIST* relacionado con la regulación de la expresión génica y condensación de la cromatina en cromosomas sexuales (Rutenberg-Schoenberg *et al*. 2016).

Los estudios epigenéticos en especies ganaderas se han centrado en la interpretación de los mecanismos moleculares que regulan la expresión de algunos genes o regiones génicas, en respuesta a factores externos como la dieta, comportamiento materno o cambios climáticos (González-Recio *et al*. 2015). Hasta el momento se han podido identificar diferentes efectos epigenéticos afectando al desarrollo de las glándulas mamarias (Devinoy and Rijnkels 2010), metabolismo lipídico y adipogénesis (Fernández-Hernando *et al*. 2011; Baik *et al*. 2014; Li *et al*. 2012), regulación del desarrollo y comportamiento animal (Jin *et al*. 2014; Ibeagha-Awemu and Zhao 2015).

Al igual que en el estudio del genoma o del transcriptoma, los avances tecnológicos en secuenciación y análisis masivo de genomas también ha favorecido el estudio de los efectos epigenéticos (Figura 6). En la regulación génica mediada por ARNnc, es posible

identificar dichos elementos reguladores mediante la integración de técnicas de análisis masivo de genomas junto con datos de expresión génica, por lo que las mejoras relativas a técnicas de secuenciación como el RNA-Seq han sido de gran ayuda en la identificación *de novo* de ARN no codificantes (Atkinson *et al*. 2012; Zhao *et al*. 2016). En el caso de regulación génica mediada por metilación del ADN ha sido posible identificar perfiles de metilación a nivel genómico específicos de diferentes tipos celulares (Weber *et al*. 2005), mediante el uso de la técnica MeDIP-Seq, basada en la secuenciación masiva de fragmentos de ADN inmunoprecipitados con un anticuerpo específico para 5mC (5-metylcytosina).

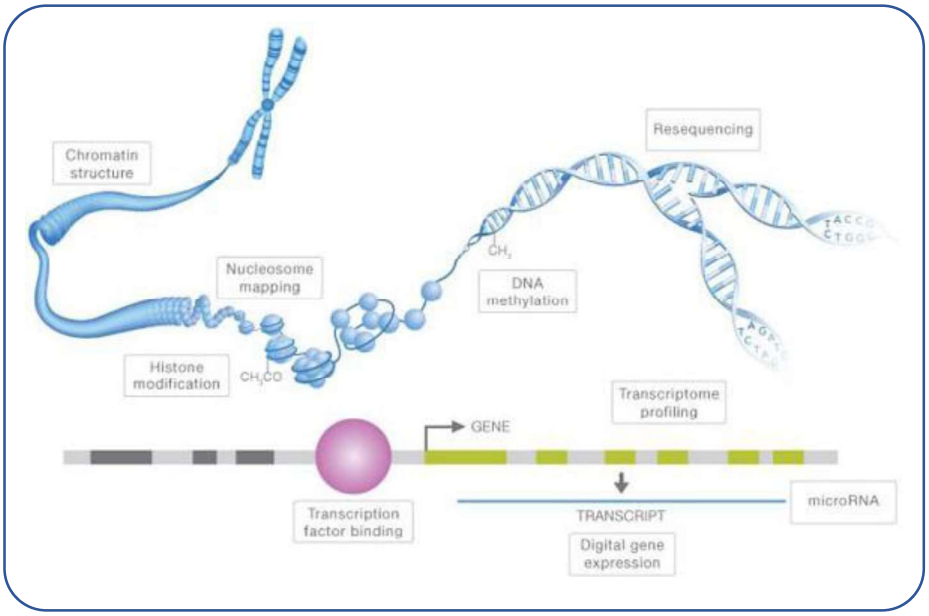

Figura 6: Posibles aproximaciones al estudio del epigenoma usando secuenciación masiva

Secuenciación masiva y el metagenoma: El estudio de la microbiota se ha establecido como campo de investigación novedoso y relevante debido a la relación entre el contenido de las comunidades microbianas y el metabolismo del hospedador, afectando a la fisiología y salud de dichos individuos (Figura 7). Los enfoques tradicionales para el análisis de la microbiota se centran en el uso de técnicas clásicas de cultivo y posterior caracterización fenotípica. Sin embargo, la aparición de las técnicas de secuenciación masiva ha provocado un impulso en la dirección de los estudios ómicos, facilitando el estudio y caracterización de la microbiota. Uno de los métodos de análisis clásico de secuenciación para el estudio filogenético de la flora intestinal en porcino es el estudio

basado en la amplificación de las regiones variables del gen ARN ribosomal 16s (Looft *et al*. 2014).

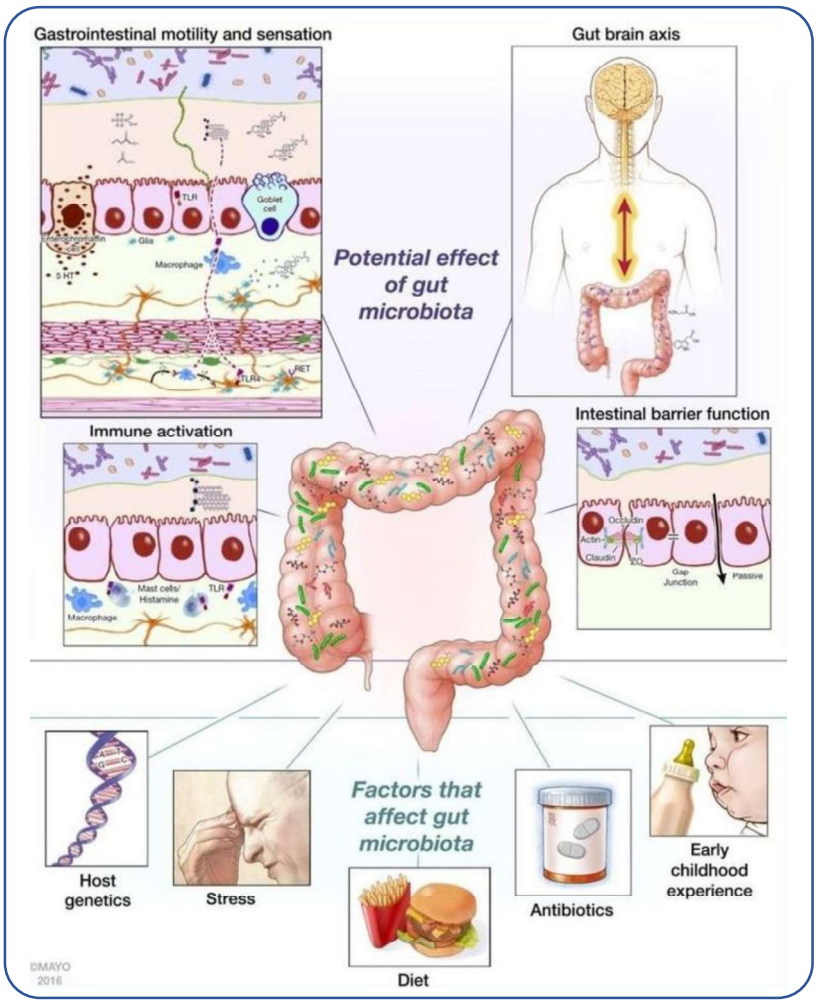

Figura 7: Microbiota gastrointestinal y mecanismos de interacción con el hospedador y ambiente

La aplicación de la secuenciación masiva en este tipo de estudios ha facilitado la caracterización clásica del gen ribosómico 16S. Sin embargo, en los últimos años se han incluido nuevas aproximaciones en la caracterización de la microbiota utilizando tecnologías que permiten la secuenciación de lecturas de mayor tamaño, facilitando la secuenciación de los genomas completos de dicha microbiota (metagenómica) como es el caso de la tecnología de PacBio y Nanopore sequencing (Frank *et al*. 2016; Brown and Clarke 2016). Además del interés en identificar la estructura filogenética de la microbiota, se plantean otros análisis como el estudio del metatranscriptoma, que se basa en la identificación de las rutas metabólicas funcionales de la microbiota, hasta el estudio

del metaproteoma, que estudia el contenido de proteínas y metabolitos expresados por la microbiota. La integración de estas diferentes aproximaciones ayuda a entender cómo responde la microbiota frente a estímulos externos, así como la interacción entre microbiota y hospedador (Deusch *et al*. 2015).

3 - Análisis de datos ómicos para la identificación de la base genética de caracteres de interés en especies ganaderas

La aplicación de tecnologías de análisis masivo de genomas descritos hasta el momento aporta gran cantidad de información de utilidad. Sin embargo, la optimización en el planteamiento es primordial para poder maximizar la información obtenida. Un correcto diseño experimental, así como la incorporación de diferentes metodologías para el análisis de datos producidos con diferentes tecnologías, proporciona mayor potencia para la obtención de resultados de interés enfocados hacia objetivos específicos.

Existen diferentes enfoques para seleccionar el material animal utilizado, como por ejemplo los cruces experimentales, que incorporan información genética de diferentes razas comerciales, o la comparación de individuos pertenecientes a grupos fenotípicamente extremos. En porcino, el diseño más utilizado hasta el momento para la detección de QTL por ligamiento son las poblaciones F2 procedentes del cruce entre líneas, poblaciones o razas divergentes para los caracteres de interés, generándose así grupos de individuos donde se suponen alelos alternativos para los QTL (Choi *et al*. 2010). A partir de este, se puede plantear un diseño más fino seleccionando dentro de cada cruce los individuos más divergentes para los caracteres que se están analizando. Este es el caso de la selección de grupos por muestreo de fenotipos extremos, divergentes para un carácter específico. Esta aproximación tiene una larga historia en estudios de desequilibrio de ligamiento, pero también han sido aplicadas para identificar tanto variaciones genómicas (Li *et al*. 2014), así como diferencias a nivel de transcriptoma relacionados con un carácter específico (Chen *et al*. 2011; Zhang *et al*. 2015; Ramayo-Caldas *et al*. 2012; Perez-Montarelo *et al*. 2014). Sin embargo, estas poblaciones son menos potentes en análisis de asociación de genomas completos, donde es más difícil determinar intervalos de confianza menores para los QTL, dificultando así la identificación de QTNs, debido al alto desequilibrio de ligamiento existente (Korte and Farlow 2013).

A su vez se pueden seguir diferentes estrategias para el análisis de la base genética de caracteres de interés a partir de datos ómicos que permiten obtener resultados complementarios a partir de un mismo material experimental.

A continuación, se exponen algunas de las más extendidas:

3.1 Identificación de QTL por ligamiento

Una de las estrategias de análisis clásicas más extendidas es la detección de QTL, basada en el ligamiento entre la mutación causal y los marcadores adyacentes, que requieren de mapas genéticos o de ligamiento desarrollados con información de marcadores genéticos. Los primeros mapas de ligamiento desarrollados en porcino empleaban marcadores moleculares tipo elementos cortos dispersos (SINEs) (Ellegren *et al*. 1994) y microsatélites (Marklund *et al*. 1996; Rohrer *et al*. 1996). El primer trabajo llevado a cabo para la identificación de QTL en porcino fue desarrollado sobre un cruce de jabalí x Large White, identificando QTL en el cromosoma SSC4 asociados con crecimiento, deposición grasa y longitud del intestino grueso (Andersson *et al*. 1994). A partir de estos primeros resultados multitud de estudios se han centrado en la identificación de QTL asociados con caracteres productivos en porcino, todos ellos recogidos en la base de datos pública PigQTLdb (Hu *et al*. 2005) (Tabla 3).

Uno de los cambios más significativos en este tipo de estudios ha sido la aparición de las plataformas de genotipado masivo, que han permitido la utilización masiva de marcadores de tipo SNPs, facilitando la construcción de mapas de ligamiento de alta densidad (Muñoz *et al*. 2012; Tortereau *et al*. 2012; Fernández *et al*. 2014) y llevar a cabo estudios de mapeo fino de QTL por ligamiento (Fernández *et al*. 2012).

3.2. Estudios de asociación de genoma completo (GWAS)

La principal aproximación que ha surgido con el desarrollo de los chips de genotipado masivo ha sido el uso de análisis de asociación de genoma completo (GWAS), cuyo objetivo principal es la utilización de un panel de marcadores denso distribuido a lo largo de todo el genoma, para detectar variantes del genoma asociadas con un carácter fenotípico de interés (Goddar *et al*. 2009). Este método se plantea como una alternativa muy extendida para identificar regiones asociadas con caracteres de interés o regiones QTL (Hill 2012; Sun *et al*. 2015). Aunque los estudios GWAS están enfocados principalmente al estudio de caracteres fenotípicos, ésta no es la única aplicación posible

de esta técnica, pudiéndose llevar a cabo estudios de genética genómica (Breitling *et al*. 2008), cuyo objetivo es la identificación de QTL para caracteres moleculares, como la expresión génica (eGWAS, eQTL), que aproximan la variación genética a caracteres fisiológicos, por lo que se denominan caracteres intermedios (Williams *et al*. 2007). Estudios previos en humanos han confirmado la validez de este tipo de aproximaciones en la identificación de variantes asociadas con enfermedades complejas (Kodama *et al*. 2012; Zou *et al*. 2012), aunque también se han trasladado a especies ganaderas en menor medida, enfocado a la salud animal (Grigoryev *et al*. 2013) y caracteres productivos (Gusev *et al*. 2016; Puig-Oliveras *et al*. 2016).

Hasta la fecha (abril 2017) la información generada en todos estos estudios de identificación de QTL en porcino asciende a 16.506 QTL, asociados con 626 caracteres a partir de 566 publicaciones (Tabla 3), incluyendo QTL por ligamiento y asociación genómica.

Tabla 3: QTL contenidos en en PigQTLdb

Tipo de Carácter	Numero de QTL
Calidad de Carne y Canal	8.974
Salud	2.925
Producción	1.782
Reproducción	1.610
Rasgos Externos	1.215
Total	16.506

3.3 Huella de la selección

Esta aproximación se basa en el estudio de las diferencias genéticas derivadas de la presión selectiva y se lleva a cabo mediante la comparación de genomas de razas o poblaciones seleccionadas para un carácter o conjunto de caracteres frente poblaciones a no seleccionadas, pudiéndose identificar patrones de variación genética asociadas a dichos caracteres, diferentes a los esperados bajo neutralidad. Los primeros estudios para la identificación de la huella de la selección empleaban diferentes estadísticos tales como el F_{ST}, que permite identificar diferencias alélicas entre poblaciones sometidas a diferentes presiones selectivas en marcadores genéticos específicos, y el Tajima's D, disminución de la diversidad genética en determinadas regiones del genoma, derivada de la conservación, por desequilibrio de ligamiento, de las regiones adyacentes a un alelo

seleccionado de forma positiva. La aparición de las tecnologías de análisis masivo del genoma ha permitido la adaptación de este tipo de análisis para la identificación de la huella de selección a nivel de genoma completo, utilizando diferentes marcadores moleculares como los SNPs o CNVs. Otra de las metodologías más utilizadas se centra en el estudio de haplotipos, basada en el modelo EHH (*Extended Haplotype Homozygosity*), utilizando estadísticos como el iHS (*Integrated Haplotype Homozygosity*) que permiten analizar los niveles de homocigosidad de haplotipos alrededor de un marcador genómico, pudiéndose identificar regiones donde la disminución de la homocigosidad es menor de lo esperado, asociada con la presión de selección en dicho punto (Cadzow *et al*. 2014).

3.4 Identificación de expresión génica diferencial

Los estudios de expresión génica diferencial informan acerca de aquellos genes que están regulados positiva o negativamente en función de una condición determinada. Una de las aproximaciones más empleadas para entender la base genética de caracteres de interés en producción animal es la comparación del transcriptoma de animales divergentes para el/los caracteres (Romero *et al*. 2012). Estos resultados pueden aportar información sobre alteraciones en rutas génicas conocidas, proporcionando información relevante para entender los mecanismos que regulan esos caracteres. Sin embargo, con la aplicación de técnicas de análisis masivo para este propósito, se pueden obtener resultados sobre rutas génicas o interacciones no descritas hasta el momento, pudiéndose identificar nuevos genes candidatos. Además, los resultados que brindan los estudios de RNA-Seq pueden ir más allá, permitiendo la identificación no solo de potenciales genes candidatos, sino de nuevas isoformas cuya expresión se vea afectada, o incluso la identificación de potenciales mutaciones causales que estén regulando la expresión de dichos genes (Ozsolak and Milos, 2011).

4 - Herramientas bioinformáticas

Con toda la información obtenida mediante las tecnologías de análisis masivos, surge la necesidad de tener un gran abanico de herramientas que permitan visualizar, editar y analizar de forma efectiva toda esta información. Este tipo de herramientas bioinformáticas son muy variadas en función de los requerimientos, datos y recursos computacionales disponibles, que van desde simples herramientas de comando que

pueden ejecutarse en un PC, a herramientas más complejas que tienen grande requerimientos computacionales, servicios web y compañías bioinformáticas de análisis.

Las diferentes herramientas informáticas pueden dividirse en herramientas de análisis de datos, que por lo general requieren de una mayor capacidad computacional, y herramientas de interpretación, que permiten analizar los resultados obtenidos en estos análisis, con el objetivo de predecir o estudiar los efectos funcionales/biológicos /fisiológicos relacionados con dichos resultados.

4.1 Herramientas bioinformáticas de análisis de datos masivos

Para analizar datos de secuenciación masiva, al tratarse de una metodología emergente, se ha propiciado la creación de una gran cantidad de herramientas. Se puede hacer una diferenciación entre herramientas de acceso libre y programas comerciales. Los segundos tienden a ser desarrolladas con el objetivo de integrar diferentes herramientas de análisis y facilitar su manejo incorporando interfaz gráficas, como es el caso de CLC Genomic Workbench (www.clcbio.com), que incluye herramientas para secuenciación masiva de genomas, RNA-Seq, identificación de polimorfismos, estudios de expresión, etc. Los programas de acceso libre suelen ser herramientas con un propósito más específico, pudiendo tratarse de programas únicos, o un conjunto de programas que comparten una misma utilidad, y que se desarrollan con la idea de una continua evolución mediada por la comunidad científica. Existen gran cantidad de este tipo de herramientas, pudiéndose agrupar por su función, en:

Herramientas de mapeo de lecturas procedentes de secuenciación de genomas como Bowtie y Bowtie 2 (Langmead *et al*. 2009; Langmead and Salzberg 2012) así como datos de RNA-Seq, como es el caso de TopHat (Trapnell *et al*. 2009) que utilizan las primeras, para el análisis de transcritos, que muestran características diferentes al ADN.

Herramientas para el análisis de expresión diferencial, que analizan datos de RNA-Seq, como Cufflinks, que incluye diferentes utilidades para el ensamblado de transcritos, cuantificación de niveles y diferencias de expresión (Cuffdiff) o identificación de nuevas isoformas expresadas (CuffCompare) (Trapnell *et al*. 2012), o DESeq, una herramienta basada en entorno de R, enfocado en el análisis de datos de secuenciación masiva, como RNA-Seq o ChiP-Seq para la identificación de diferencias de expresión (Anders *et al*. 2010).

Herramientas para la identificación de variantes genómicas, donde existe cierta distinción entre herramientas más potentes para la identificación de SNPs (SAMTools (Li *et al*. 2009), o de indels (GATK, software.broadinstitute.org/gatk/) o de variantes estructurales tipo CNV (CNVSeq, FREEC, readDepth, CNVnator, SegSeq, Duan *et al*. 2013).

Herramientas bioinformáticas-estadísticas: Las herramientas más clásicas de análisis de datos de marcadores han tenido que adaptarse a esta nueva era, como es el caso de los programas para la construcción de mapas de ligamiento como el CRI-MAP (Green *et al*, 1990) o para estudios de asociación como Qxpak (Pérez-Enciso and Misztal 2011), adaptándose a tamaños de pedigrees mayores y sobre todo al mayor número de marcadores genéticos disponibles. Además han surgido nuevas herramientas ampliamente usadas como es el caso de PLINK, que permite editar y procesar grandes cantidades de genotipos de una manera eficiente (Chang *et al*. 2015), herramientas con objetivos más específicos como la reconstrucción de haplotipos y estimación de la tasa de recombinación como Haploview (Barrett *et al.* 2005) y PHASE (Stephens *et al*. 2001) o herramientas que aportan soporte para una multitud de análisis estadísticos a nivel de genoma, adaptados a la gran cantidad de datos disponibles y maximizando el consumo computacional, como es el caso de GenABEL (Karssen *et al*. 2016).

4.2 Herramientas bioinformáticas para la interpretación de resultados

Existe una gran cantidad de herramientas que permiten interpretar los resultados obtenidos en estos estudios, desde el análisis de la función génica, hasta la predicción de los efectos asociados a variantes génicas. Algunas de las más utilizadas son:

- STRING: Permite la extracción de información de interacciones génicas y proteicas, a partir de información experimental y de predicciones basadas en minería de datos y transferencia de interacciones por ontología a partir de especies modelo (Szklarczyk *et al*. 2015).
- VarElect: Permite priorizar listas de genes en función de información relacionada con fenotipos de interés, utilizando minería de datos en diferentes bases de datos (Stelzer *et al*. 2016).
- VeP Ensembl: Permite determinar el efecto de variantes génicas (SNPs, INDELs, CNVs, etc) a partir de secuencias de genes, transcritos y proteínas.

- Babelomics: Permite analizar expresión génica, variación genómica y caracterización funcional (Alonso *et al*. 2015). Una de las herramientas más utilizadas en este paquete, para el estudio de enriquecimiento funcional a partir de un set de genes es FatiGO (Al-Shahrour *et al*. 2004).
- PredictProtein: Permite predecir estructura secundaria y funciones proteícas a partir de secuencias de aminoácidos, permitiendo identificar el efecto de mutaciones a nivel de proteína (Yachdav *et al*. 2014).
- SNAP2 y SIFT: Permiten predecir efecto de variantes genéticas y el posible efecto funcional de sustituciones de amino ácidos en secuencias proteicas (Hecht *et al*. 2015; Kumar *et al*. 2009).
- RegRNA: Herramienta para la identificación de motivos funcionales de ARN a partir de secuencias de ARN, permitiendo estudiar el efecto de mutaciones genómicas a nivel de estabilidad y disponibilidad de la molécula de ARN (Chang *et al*. 2013)
- MEME SUITE: Se trata de un paquete de herramienta que permite estudiar motivos a nivel de ADN, ARN y proteína (Bailey *et al*. 2009). Algunas de estas herramientas utilizadas en este trabajo son: MEME que permite identificar motivos de ADN a partir de secuencia (Bailey and Elkan 1994), GOMo que permite analizar enriquecimiento funcional (Términos GO) de los genes donde se encuentra un motivo (Buske *et al*. 2010) y TOMTOM que permite identificar motivos similares en las bases de datos (Gupta *et al*. 2007).

5 - Antecedentes del presente trabajo

Los antecedentes al presente trabajo se remontan a 1996 donde la colaboración de los grupos de investigación de la Universitat Autónoma de Barcelona (UAB), el Institut de Recerca i Tecnologia Agroalimentàries (IRTA) y el Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) permitió generar el cruce experimental Ibérico x Landrace, denominado IBMAP (Figura 8). Primero se generó una población F2 y posteriormente se obtuvieron generaciones F3 y diversas generaciones de retrocruces. Todo este material animal, del que se cuenta con registros de múltiples caracteres productivos y de calidad de carne relacionados con crecimiento, deposición grasa, conformación, rendimientos, composición de ácidos grasos en grasa y músculo se generó con el objetivo de identificar la base genética de este tipo de caracteres. Además

del último material generado, retrocruces F1 (Ibérico x Landrace) x Landrace, F1 (Ibérico x Duroc) x Duroc y F1 (Ibérico x Pietrain) x Pietrain), se han podido coleccionar biopsias de tejidos clave (hígado, hipotálamo, grasa subcutánea y músculo *Longissimus dorsi*) que han permitido complementar estudios funcionales y de expresión génica.

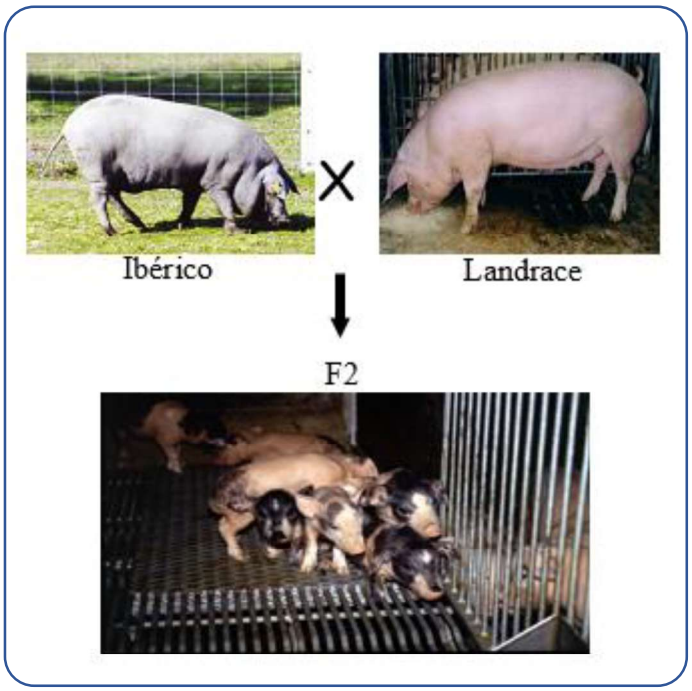

Figura 8: Imágenes de los parentales e individuos F2 del cruce experimental Ibérico x Landrace (Población IBMAP original)

Sobre este material se han venido aplicando diversas estrategias de análisis desde QTL de ligamiento con microsatélites (Pérez-Enciso and Varona 2000; Óvilo *et al*. 2000; Varona *et al*. 2002; Óvilo *et al*. 2002; Pérez-Enciso *et al*. 2002; Clop *et al*. 2002; Clop *et al*. 2003) pasando por el análisis de genes candidato (Amills *et al*. 2005; Óvilo *et al*. 2006; Óvilo *et al* 2010; Kim *et al*. 2006; Mercadé *et al*. 2006a; Mercadé *et al*. 2006b; Estellé *et al*. 2006; Muñoz *et al*. 2007; Estellé *et al*. 2009a; Estellé *et al*. 2009b; Corominas *et al*. 2012; Pérez-Montarelo, *et al*. 2012; Corominas *et al*. 2013b; Muñoz *et al*. 2013a; Pérez-Montarelo *et al*. 2013; Revilla *et al*. 2014), QTL de ligamiento y GWAS con chips de alta densidad de SNPs (Fernández *et al*. 2012, Ramayo-Caldas *et al*. 2012a; Muñoz *et al*. 2013b; Ramayo-Caldas *et al*. 2014; Fernández *et al*. 2014) y microarrays de expresión génica (Pena *et al*. 2013; Muñoz *et al*. 2013b). Fruto de todo este trabajo se han identificado más de 30 QTL con efectos relevantes para crecimiento, deposición grasa, composición de ácidos grasos y contenido en colágeno principalmente en los cromosomas

SSC4, SSC6, SSC8, SSC12 y SSCX, e identificado potentes mutaciones causales principalmente en los genes *LEPR* (Pérez-Montarelo *et al*. 2012) y *ELOVL6* (Corominas *et al*. 2013b) y se han validado los efectos de otras mutaciones causales en los genes *IGF2* (Estellé *et al*. 2005), *MTTP* (Estelle *et al*. 2009), *RYR1* (Fernández *et al*.en proceso) y *SCD* (Fernández *et al*. en revisión).

Más recientemente se ha incorporado a estos análisis la secuenciación de genomas y en mayor número de trasncriptomas completos, basados en la comparación de los niveles de expresión génica en hígado, grasa, músculo e hipotálamo de animales de los retrocruces con fenotipos extremos para caracteres de crecimiento, deposición grasa y rendimientos (Pérez-Montarelo *et al*. 2014) o para caracteres de calidad de la carne y grasa (Ramayo-Caldas *et al*. 2012b; Corominas *et al*. 2013a; Puig-Oliveras *et al*. 2014).

A partir de todos estos recursos, datos y resultados generados principalmente en los retrocruces F1 (Ibérico x Landrace) x Landrace, F1 (Ibérico x Duroc) x Duroc y F1 (Ibérico x Pietrain) x Pietrain se ha llevado a cabo la presente tesis doctoral.

Objetivos

El objetivo principal del presente trabajo de tesis doctoral ha sido contribuir al conocimiento de la base genética de caracteres de crecimiento, deposición grasa y rendimiento de piezas nobles explotando diferentes fuentes de información genómica y transcriptómica y explorando aspectos metodológicos. El objetivo final ha sido identificar genes y mutaciones candidatas a regular la expresión de este tipo de caracteres.

Este objetivo principal se ha dividido en tres objetivos parciales que han dado lugar a tres estudios, dos de ellos ya publicados en revistas SCI de alto impacto en el área de la genética y producción animal y otro que será enviado a otra revista del mismo nivel.

Objetivo 1) Identificar potenciales mutaciones causales para caracteres productivos en porcino a partir del análisis de datos de secuenciación masiva de ARN (RNA-Seq)

Martínez-Montes AM, Fernández A, Pérez-Montarelo D, Alves E, Benítez RM, Nuñez Y, Óvilo C, Ibañez-Escriche N, Folch JM, Fernández AI. 2017. Using RNA-Seq SNP data to reveal potential causal mutations related to pig production traits and RNA editing. Anim Genet. 2017; 48(2):151-165. doi: 10.1111/age.12507. Epub 2016 Sep 18.

Objetivo 2) Descifrar el funcionamiento de la regulación de los genes porcinos que influyen en el crecimiento, deposición grasa y rendimiento de piezas nobles a través de genética genómica.

Martínez-Montes AM, Muiños-Bühl A, Fernández A, Folch JM, Ibáñez-Escriche N, Fernández AI. Deciphering the regulation of porcine genes influencing growth, fatness and yield-related traits through genetical genomics. Mamm Genome. 2016 Dec 10. [Epub ahead of print]

Objetivo 3) Identificar QTL comunes y específicos en tres fondos genéticos distintos basados en cerdo Ibérico usando análisis de asociación de genomas completos.

Martínez-Montes AM, Fernández A, Muñoz M, Noguera JL, Folch JM, Fernández AI Using genome wide association studies to identify common and specific QTL in three different genetic backgrounds based on Iberian pig breed. PlosOne [en preparación]

Artículo I

Using RNA-Seq SNP data to reveal potential causal mutations related to pig production traits and RNA editing

Martínez-Montes AM, Fernández A, Pérez-Montarelo D, Alves E, Benítez RM, Nuñez Y, Óvilo C, Ibañez-Escriche N, Folch JM, Fernández AI. 2017. Using RNA-Seq SNP data to reveal potential causal mutations related to pig production traits and RNA editing. Anim Genet. 2017; 48(2):151-165. doi: 10.1111/age.12507. Epub 2016 Sep 18.

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Using RNA-Seq SNP data to reveal potential causal mutations related to pig production traits and RNA editing

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Summary

RNA-Seq technology is widely used in quantitative gene expression studies and identification of non-annotated transcripts. However this technology also can be used for polymorphism detection and RNA editing in transcribed regions in an efficient and cost-effective way. This study used SNP data from an RNA-Seq assay to identify genes and mutations underlying production trait variations in an experimental pig population. The hypothalamic and hepatic transcriptomes of nine extreme animals for growth and fatness from an (Iberian × Landrace) × Landrace backcross were analyzed by RNA-Seq methodology, and SNP calling was conducted. More than 125 000 single nucleotide variants (SNVs) were identified in each tissue, and 78% were considered to be potential SNPs, those SNVs segregating in the context of this study. Potential informative SNPs were detected by considering those showing a homozygous or heterozygous genotype in one extreme group and the alternative genotype in the other group. In this way, 4396 and 1862 informative SNPs were detected in hypothalamus and liver respectively. Out of the 32 SNPs selected for validation, 25 (80%) were confirmed as actual SNPs. Association analyses for growth, fatness and premium cut yields with 19 selected SNPs were carried out, and four potential causal genes (*RETSAT*, *COPA*, *RNMT* and *PALMD*) were identified. Interestingly, new RNA editing modifications were detected and validated for the *NR3C1*:g.102797 (ss1985401074) and *ACSM2B*:g.13374 (ss1985401075) positions and for the *COG3*:g3.4525 (ss1985401087) modification previously identified across vertebrates, which could lead to phenotypic variation and should be further investigated.

Keywords association, fatness, Iberian pig, premium cut yield, transcriptom

Introduction

The recent availability of massive sequencing technologies provides new tools for the search of causal genes and mutations. Several approaches, such as whole genome sequencing, exome capture and sequencing, chromatin immunoprecipitation sequencing and transcriptome sequencing, have been developed to answer different biological questions (Bai *et al.* 2012). In particular, RNA sequencing (RNA-Seq) technology is largely used in quantitative gene expression studies as a source of biological information to support the identification of causal mutations underlying the variation of complex traits (Hudson *et al.* 2012). RNA-seq methodology allows for a comprehensive analysis and quantification of all RNA types expressed in tissues or cells, including mRNA, non-coding RNA and small RNA (Wang *et al.* 2009). In comparison with gene expression microarrays, RNA-seq technology is able to detect transcripts expressed at low levels and alternative isoforms (Ferraz *et al.* 2008; Trapnell *et al.* 2009). During the last few years, the RNA-seq method has also been employed with farm animals and has helped in the selection of candidate genes related to important traits through the comparison of global gene expression profiles between groups of animals that differ in specific traits (i.e. Ramayo-Caldas *et al.* 2012; Pérez-Montarelo *et al.* 2014; Wang *et al.* 2015; Zhang *et al.* 2015).

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Besides allowing the detection of differentially expressed genes, RNA-Seq also enables the identification of previously non-annotated transcripts. Moreover, RNA-Seq technology can be exploited as a method to detect polymorphisms in transcribed regions in an efficient and cost-effective way (Chepelev *et al.* 2009; Cirulli *et al.* 2010). In spite of all this, the use of RNA-Seq SNP data to identify candidate genes and mutations in farm animals has not been widely applied (Koltes *et al.* 2015). To date, only two studies using RNA-Seq for polymorphisms identification in transcribed regions have been reported (Cánovas *et al.* 2010; Sharma *et al.* 2012), but further associations and functional interpretation have not been conducted.

Even more, the use of RNA-Seq technology allows the detection of differential allelic expression and post-transcriptional modifications such as RNA editing (Frésard *et al.* 2015). RNA editing is a posttranscriptional mechanism that generates new transcripts from a limited number of genes in the genome and consists of the chemical alteration of nucleotide bases of RNA molecules (Venø *et al.* 2012). RNA editing may result in a nucleotide modification, insertion, deletion or substitution in the RNA sequence and may occur in various types of RNA, whether coding or not (Knoop 2011). To the best of our knowledge, only human, mouse, rat, sheep (Shah *et al.* 2009; Caiment *et al.* 2010; Danecek *et al.* 2012; Holmes *et al.* 2013) and recently chicken (Frésard *et al.* 2015) studies have identified RNA editing events, except for the known *apolipoprotein B* (*APOB*) gene (Greeve *et al.* 1993).

The main aim of the current study was to use SNP data from a RNA-Seq assay to successfully identify polymorphisms underlying production trait variations in the IBMAP (Iberian × Landrace) experimental porcine population. RNA-editing phenomena were detected from RNA-Seq SNP data and validated for some interesting genes.

Materials and methods

Animal selection, RNA processing and sequencing

The animal material used in the present study was derived from a backcross generated from the IBMAP population. The IBMAP pig population (Iberian × Landrace crosses, including F2, F3 generations and backcrosses) was generated to identify QTL, genes and causal mutations responsible for the variation in production and meat quality traits in pigs, given the remarkable differences existing for such traits between the Iberian and Landrace parental lines (Serra *et al.* 1998). Results of QTL scans revealed the existence of QTL for growth, fatness and premium cut yields in porcine chromosomes SSC1, SSC2, SSC4, SSC5, SSC6, SSC9, SSC11, SSC13, SSC14, SSC17 and SSCX (Óvilo *et al.* 2000, 2002; Varona *et al.* 2002; Mercadé *et al.* 2005; Fernández *et al.* 2012, 2014a,b).

The backcross was generated from three Iberian boars mated with 30 Landrace sows (F0) to produce 70 F1 animals. Five F1 boars were mated with 25 Landrace sows, and 187 backcross animals were obtained. All pigs were grown on an experimental farm under standard conditions. Animal manipulations were performed according to the Spanish Policy for Animal Protection RD1201/05, which meets European Union Directive 86/609 concerning the protection of animals used in experimentation. The animals were slaughtered at an approximate age of 175 days. Phenotypic traits related to growth, fatness and premium cut yields were measured in all backcrossed animals as previously described (Fernández *et al.* 2012) (Table 1).

The most extreme animals for growth and fatness were selected as described in Pérez-Montarelo *et al.* (2014). Briefly, a principal components analysis of the backcrossed animals was performed according to four indicators for growth and fatness traits. The 10 male pigs from the same slaughter batch with the most extreme phenotypes, according to the first principal component, were selected for this study and divided into two groups. The five males showing the highest values of growth and fatness indicators were assigned to the High (H) group, and the five males showing the lowest values for these traits to the Low (L) group. The mean values of the four indicators in the H and L groups were respectively 0.92–0.74 kg/day average daily gain, 16.2–11.6 mm of backfat thickness, 12.6–16.7% of C18:2($n-6$) in backfat and 8.1–11.9% of C18:2 ($n-6$) in intramuscular fat.

Hypothalamic and hepatic tissue samples from the 10 selected animals were collected at slaughter, immediately frozen in liquid nitrogen and stored at −80 °C until analyzed. Total RNA was extracted using the Ribopure kit (Ambion) to produce high-quality total RNA, following the manufacturer's recommendations, and quantified using a NanoDrop-100 spectrophotometer. The integrity of the RNA was assessed using an Agilent 2100 Bioanalyzer. The RNA integrity value of the samples ranged from 7.1 to 8.1. Paired-end libraries with fragments of 300 bp were prepared using the TruSeq RNA Sample Prep Kit v2 (Illumina Inc.) for each sample. Multiplex sequencing of the libraries was performed on an Illumina Hi-Seq 2000 (Fasteris SA) with three samples per lane at the Centro Nacional de Análisis Genómico, according to the manufacturer's instructions, generating paired-end reads of 75 bp.

Table 1 Phenotypic traits recorded from backcrossed animals of the IBMAP experimental population.

Trait description	*n*	Mean	SD
Body weight at 150 days (kg)	159	79.13	10.49
Backfat thickness at 75 kg	159	12.69	1.50
Intramuscular fat	124	2.06	0.70
Ham weight (kg)	154	10.22	1.39
Shoulders weight (kg)	154	5.43	0.80
Bone-in-loin weight (kg)	153	7.09	1.03

The raw sequence data have been deposited in the GEO expression database under accession nos. GSE51968 and GSE75850.

RNAseq data analysis

Quality of the raw sequencing data was determined with FASTQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). TRIM GALORE (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) was used to qualitatively trim the data using default settings and to remove the sequencing adaptors and poly A and T tails (stringency of 6 bp), keeping only paired-end reads for which both pairs were longer than 40 bp. One of the 10 samples was discarded for further analyzes due to quality problems (Pérez-Montarelo *et al.* 2014).

SNP calling

The RNA-seq data filtering, mapping and polymorphisms (single nucleotide variant, SNV) calling was conducted with CLC GENOMICS WORKBENCH software (www.clcbio.com). Filtered reads were mapped against the pig reference genome (Sscrofa 10.2) based on Mortazavi *et al.* (2008). Mapping parameters were set at a maximum of two gaps or mismatches per read, and the distance between pairs was set to 50 bp (inner-mean distance) and a standard deviation of 150 bp. Quality-based VARIANT DETECTION from CLC GENOMICS WORKBENCH was used to perform the SNV detection, with quality parameters set as: minimum quality of central base at 20, minimum quality of surrounding bases (3 pb) at 15, minimum coverage at 10× and a minimum variant frequency at 20%. The QUALITY-BASED VARIANT DETECTION method in CLC GENOMICS WORKBENCH is based on the neighborhood quality standard algorithm described by Altshuler *et al.* (2000) and Brockman *et al.* (2008), using the PHRED quality score surrounding the potential SNP. The SNP calling was conducted within tissue, using all reads from the same tissue for polymorphism identification. After, individual genotypes were identified for each single variant. Those SNVs segregating in the sequenced animals, heterozygous or alternative homozygous among the sequenced animals, were considered actual single nucleotide polymorphisms. SNPs showing divergent genotypes between the H and L groups (homozygous or heterozygous in one group and alternative allele homozygous in the other group, and considering absence of genotype equal to the reference allele at this position) were selected as potential informative SNPs.

Variant annotation, functional enrichment and effect predictions

The Ensembl Variation database in Biomart (www.ensembl.org/biomart) was used to annotate previously described porcine variants. Functional annotation and enrichment analysis were conducted using the FATIGO tool from BABELOMICS (Medina *et al.* 2010) and human genome annotation as a reference. Adjusted *P*-values, based on the false discovery rate (FDR) method of accounting for multiple testing (Benjamini & Hochberg 1995), were used to identify Gene Ontology enrichment terms. The PREDICTPROTEIN (www.predictprotein.org) tool was used to predict amino acid change effects on protein structure and function, and REGRNA (http://regrna.mbc.nctu.edu.tw/) was used to identify changes in regulatory motifs.

SNP validation

A set of 32 SNPs were selected for validation based on functional and positional criteria and potential effect relevance. Validation was conducted by standard Sanger sequencing on cDNA synthesized from RNA from both tissues from the same animals used for the RNA-Seq assay. Primer pairs were designed from exon to exon to avoid genomic DNA amplification (Table S1). The PCR reactions were performed in a final volume of 25 μl, containing 2.5 μl of cDNA, 1 unit of Taq polymerase (Biotools), specific buffer, 2.5 mM of dNTPs and 0.5 μM of each primer. Thermocycling was carried out under the following conditions: 94 °C for 5 min, 40 cycles of 94 °C for 45 s, 60 °C for 45 s and 72 °C for 45 s, with a final extension of 72 °C for 10 min. The PCR reactions were carried out in a GeneAmp PCR System 9700 (Applied Biosystems). The PCR products were purified with the GFXTM PCR DNA purification kit (GE Healthcare), according to the manufacturer's protocol. PCR products were sequenced with both forward and reverse primers using the 3100 BigDye® Terminator v3.1 Matrix Standard in a 3730 DNA Analyzer (Applied Biosystems).

SNP genotyping and association analyses

Fourteen candidate genes – *retinol saturase* (*RETSAT*); *megakaryoblastic leukemia (translocation) 1* (*MKL1*); *serpin peptidase inhibitor, clade E* (*SERPINE1*); *synoviolin 1* (*SYVN1*); *leucyl-tRNA synthetase* (*LARS*); *coatomer protein complex subunit alpha* (*COPA*); *cell adhesion molecule 3* (*CADM3*); *ephrin-A1* (*EFNA1*); *palmdelphin* (*PALMD*); *calponin 3* (*CNN3*); *rhomboid like 2* (*RHBDL2*); *janus kinase 1* (*JAK1*); *hydroxyacid oxidase (glycolate oxidase) 1* (*HAO1*); and *RNA (guanine-7-) methyltransferase* (*RNMT*) – carrying SNPs identified in the RNA-Seq assay and subsequently validated, were selected for association analyses due to their potential role in biological processes involved in traits related to growth, fatness and premium cut yields (functional candidate genes) or their putative positions within QTL intervals (positional candidate genes).

Functional candidate genes

Polymorphisms located in seven functional candidate genes (described below) were selected due to their functional relationship with the analyzed traits. The *RETSAT* gene

encodes a retinol saturase, which promotes adipogenesis and is downregulated in obesity (Schupp *et al.* 2009). The *SERPINE1* gene encodes a serpin peptidase inhibitor that has been associated with muscling, growth, fat accretion and meat quality in pigs (Weisz *et al.* 2012). The *MKL1* gene, also known as *MRTF-A*, encodes the megakaryoblastic leukemia protein, which interacts with the transcription factor myocardin and which is a key regulator of muscle cell differentiation and remodeling (Mokalled *et al.* 2012). The *HAO1* gene encodes a hydroxyacid oxidase, which constitutes a liver-specific peroxisomal enzyme that oxidizes glycolate to glyoxylate and is a known diabetes marker (Recalcati *et al.* 2003). The *RNMT* gene (similar to *LOC100626038*) encodes a methyltransferase involved in mRNA processing, stability and translation required for cell proliferation (Aregger & Cowling 2013). The *RHBDL2* gene encodes an integral membrane protein that releases soluble growth factors by proteolytic cleavage of certain membrane-bound substrates (Pascall & Brown 2004); specifically it releases epidermal growth factor, which mediates adipocyte differentiation (Harrington *et al.* 2007). The *SYVN1* gene encodes a synovial apoptosis inhibitor that removes unfolded proteins; it is implicated together with PGC-1β in the regulation of mitochondrion number, respiration and basal energy expenditure in adipose tissue, and therefore, it determines weight and accumulation of white adipose tissue (Fujita *et al.* 2015).

Ten SNPs in these seven candidate genes were genotyped: *RETSAT*:g.3320A>C, *RETSAT*:g.*3453*A>C, *RETSAT*:g.3661in delT, *RETSAT*:g.3962A>G, *SERPINE1*:g.1249A>G, *MKL1:* g.10685T>C; *HAO1*:g.7569A>T, *RNMT*:g.185A>C, *RHBDL2:* g.19048A>G and *SYVN1*:g.2895G>T (submitted to SNPdb as ss1985400207, ss1985400206, ss1985400205, ss1985400204, ss1985400218, ss1985400213, ss1985400216, ss1985400217, ss1985400214, ss1985400201 respectively).

Positional candidate genes

Polymorphisms located in seven genes mapped within QTL intervals for the analyzed traits were selected: the *LARS* gene maps to 153 Mb on SSC2, close to the QTL for premium cut yield identified previously (Fernández *et al.* 2012). This gene encodes a leucyl-tRNA synthetase, which catalyzes the ATP-dependent ligation of L-leucine to tRNA (Leu), responsible for specific hepatopathies (Casey *et al.* 2012); the *JAK1* gene maps to 135 Mb on SSC6, within the QTL interval for backfat thickness and premium cut yields previously identified (Óvilo *et al.* 2000; Varona *et al.* 2002; Fernández *et al.* 2012) and encodes a protein-tyrosine kinase, directly implicated in insulin resistance in obese individuals (Khan *et al.* 2015). Two missense SNPs in the *LARS* gene (g.33280A>C and g.7010G>A) and two nonsense SNPs in the *JAK*1 gene (g.18273A>G and g.12288A>G) were selected for genotyping.

Five other functional gene candidates underlying the QTL effects on SSC4 for fatness and premium cut yields were also analyzed: *PALMD*:g.210G>C, *CNN3*:g.17250A>G, *COPA: g.48255C>T*, *CAMD3:*g.21844T>C and *EFNA1*:g.5633T>C (submitted to SNPdb as ss1985400203, ss1985401075, ss1985400207, ss1985400208 and ss1985401076 respectively). The *palmdelphin* (*PALMD*) gene maps to 129 Mb and encodes a protein involved in p53 phosphorylation (Dashzeveg *et al.* 2014), which is crucially involved in the development of insulin resistance through the modulation of cell apoptosis (Minamino *et al.* 2009); the *calponin 3* (*CNN3*) gene maps to 134 Mb and encodes a protein associated with growth traits in pig (Tang *et al.* 2014); the *coatomer protein complex subunit alpha* (*COPA*) gene maps to 98 Mb and encodes a coat protein responsible for the transport between the endoplasmic reticulum and Golgi compartments, which may play an important role in muscle development (Qiu *et al.* 2010); the *ephrin-A1* (*EFNA1*) gene maps to 103 Mb and encodes a protein-tyrosine kinase receptor, which is implicated in angiogenesis (Deroanne *et al.* 2003) and consequently in adipose tissue expansion (Rupnick *et al.* 2002).

The *RETSAT* (ss1985400204, ss1985400205, ss1985400206, ss1985400207), *MKL1* (ss1985400213) and *SERPINE1* (ss1985400218) SNPs were genotyped in 122 backcrossed animals with available samples and registered phenotype using Sanger sequencing. Primer pairs are described in Table S1. Additionally, (*SYVN1*) (ss1985400201), *LARS* (ss1985400202, ss1985400203), *COPA* (ss1985400208), *CADM3* (ss1985401078), *EFNA1* (ss1985400210), *PALMD* (ss1985400211), *CNN3* (ss1985400212), *RHBDL2* (ss1985400214), *JAK1* (ss1985401084, ss1985400215), *HAO1* (ss1985400216) and *RNMT* (ss1985400217) polymorphisms were genotyped using the OpenArray platform at Servei Veterinari de Genètica Molecular (Universidad Autónoma de Barcelona, Spain). Haplotypes were built using PHASE version 2.1 program (Stephens *et al.* 2001).

Association analyses of the SNPs and haplotypes with the production traits body weight at 150 days, backfat thickness at 75 kg, intramuscular fat and premium cut weights (ham, shoulder and bone-in loins) were conducted with QXPAK 5.0 software (Pérez-Enciso & Misztal 2011), using the univariate animal model:

$$y_{ijk} = S_i + B_j + bx_k + \sum_l \lambda_{lk} a_l + u_k + e_{ijk},$$

where y_{ijk} is the trait value of kth individual; S_i and B_j are the sex and batch fixed effects respectively; b is the regression coefficient on carcass weight (included only for intramuscular fat and premium cut weights); a_l is the additive effect of the SNP or haplotype; λ_{lk} is an indicator variable related to the number of copies (0, 1 or 2) of the lth allele (one in the analysis of SNPs); u_k is the infinitesimal genetic effect of the kth individual (treated as random with covariance matrix $\mathbf{A}\,\sigma_u^2$, A being the numerator relationship matrix); and e_{ijk} is the

random residual term. Dominance effect was not included in the model because it was not significant. Strict Bonferroni correction (α/SNP number) was applied to correct for multiple tests ($0.05/19 = 2.6 \times 10^{-3}$).

Gene expression analyses

Differential gene expression conditional on SNP genotypes that showed significant associations with production traits were evaluated here using the RNA-Seq expression data (Fernández *et al.* 2014a,b; Pérez-Montarelo *et al.* 2014). Transcripts were assembled and quantified in RPKM (reads per kilobase of transcript per million mapped) by CLC GENOMICS WORKBENCH.

Validation of gene expression differences for *RETSAT* and *PALMD* on hepatic tissue were conducted by quantitative PCR (qPCR) in a larger sample set; hepatic samples were from 31 males from the same slaughter batch. The cDNA synthesis was performed using Superscript II enzyme (Invitrogen). Relative transcript quantification was performed on 384 plates using the LightCycler®480 Real-Time PCR System (Roche Diagnostic). Real-time qPCR reactions were performed in a total volume of 20 μl containing 2.5 μl of cDNA (1/20 dilution), 10 μl of Roche LightCycler mix and 0.5 μM of primer pairs (Table S1). All points and samples were run in triplicate as technical replicates, and dissociation curves were analyzed for each individual replicate. *Actin, beta* (*ACTB*) and *TATA-box binding protein* (*TBP*) were used as control genes.

Gene expression data were normalized by the endogenous genes using GENORM software (http:/medgen.ugent.be/~jvdesomp/genorm) and analyzed using a general linear mixed model including the additive effect of the SNP or haplotypes and a random litter effect accounting for the correlations among the records of full sibs.

RNA editing validation

A subset of three SNPs showing different genotypes conditional on the analyzed tissue (hypothalamus or liver) was also validated on genomic DNA (gDNA). Primer pairs were designed (Table S1), and amplification and sequencing were conducted as mentioned above. Furthermore, pyrosequencing protocols (Table S1) were designed to validate the genotype differences between DNA sources (gDNA or cDNA). In addition, gDNA and hypothalamic RNA samples from three pure Iberian and three Iberian × Large White pigs were also added to the validation step.

Results

SNP detection

A total of 839 and 877 millions of paired reads were obtained from hypothalamus and liver RNA-Seq data respectively. After filtering by quality and mapping against the reference genome Sscrofa 10.2, 66% and 77% of the reads matched the reference sequence respectively, and 50% corresponded to annotated genes.

The variant detection analysis revealed a total of 125 488 SNVs in hypothalamus and 125 163 SNVs in liver (Table 2), and 45% of the SNVs were identified in both tissues, giving a total of 192 143 SNVs that were detected against the reference genome in this study. Those SNVs segregating in the sequenced animals were considered as actual SNPs: 97 427 SNPS identified in hypothalamus and 99 290 SNPs in liver. Approximately 65–70% of these SNPs were located in exonic regions already annotated, and roughly 10% of them corresponded to missense SNPs (Table 2). A total of 4396 informative SNPs were detected in hypothalamus and 1862 in liver, including 331 SNPs that were detected in both tissues. Informative SNPs were considered those showing a homozygous or heterozygous genotype in one group and the alternative genotype in the other group.

The enrichment of biological processes affected by the genes that contained the identified SNPs in both tissues (Table 3) revealed that the same general terms – organic acid carboxylic acid, cellular amino acid and derivative and lipid metabolisms – were enriched in both tissues. However, the regulation of the developmental process term was specifically enriched in hypothalamus, in accordance with the fact that it is the main tissue implicated in hormonal coordination, complex patterns of neuroendocrine outputs and homeostatic mechanisms during development (Swaab *et al.* 2001).

The distribution of the detected SNPs along the porcine chromosomes is shown in Fig. 1. The proportions of SNPs

Table 2 Summary of SNVs and SNPs identified in hypothalamus and liver by RNA-Seq from the two divergent groups for growth and fatness.

	Gene	Exon	Coding region	Missense	$n > 2$[1]
Hypothalamus					
SNVs	125 488	84 941	30 472	9588	98 869
SNPs[2]	97 427	63 252	24 527	7310	77 548
Potential informative SNPs[3]	4396	3277	835	242	4396
Liver					
SNVs	125 163	93 560	23 605	7707	104 621
SNPs[2]	99 290	72 792	19 092	5994	76 327
Potential informative SNPs[3]	1862	1341	383	132	1862

[1]SNPs identified in more than two of the sequenced animals.
[2]SNVs segregating, at least two animals heterozygous or homozygous for the alternative allele, were considered SNPs.
[3]Those SNPs for which the animals were homozygous or heterozygous in one group and alternative allele homozygous in the other group were considered potential informative SNPs.

per chromosome were compared with those obtained from the Ensembl Variation Database (Ensembl Variation 82; *Sus scrofa* Short Variants 10.2). Both distributions were similar (Fig. 1a), but differences were observed when focusing on the potential informative SNPs (P-value = 10^{-4}), especially for some chromosomes such as SSC6, where the number of SNPs was much higher than in the annotated results in the Ensembl database (Fig. 1b).

Table 3 Summary of the Gene Ontology enrichment terms for the genes containing the SNPs identified in hypothalamus and liver by RNA-Seq.

Gene Ontology terms	No. of genes	Adjusted P-values[1]
Hypothalamus		
Organic acid metabolic process	80	3.69×10^{-4}
Regulation of developmental process	110	3.96×10^{-4}
Carboxylic acid metabolic process	78	6.29×10^{-4}
Celular amino acid and derivative metabolic process	41	6.82×10^{-4}
Lipid metabolic process	105	9.07×10^{-4}
Liver		
Organic acid metabolic process	57	4.64×10^{-6}
Carboxylic acid metabolic process	57	4.64×10^{-6}
Lipid metabolic process	72	4.64×10^{-6}
Celular lipid metabolic process	62	6.44×10^{-6}
Amine metabolic process	41	3.04×10^{-4}

[1]Adjusted P-values based on false discovery rate method of accounting for multiple testing (Benjamini & Hochberg 1995).

A set of 32 SNPs (Table 4) was selected for validation. These SNPs met one or more of the following criteria: involvement in biological processes related to the analyzed traits, inducing amino acid changes, location on QTL regions previously described (Óvilo *et al.* 2000; Varona *et al.* 2002; Fernández *et al.* 2012, 2014a,b) and genotype differences between tissues. The sequencing of cDNA from the same hypothalamic and hepatic samples confirmed that 25 out of the 32 detected SNPs were actual SNPs, representing 80% of the total number of tested SNPs. From the remaining SNPs, six were false positives and one of them could not be tested due to unsuccessful amplification (Table 4).

Association studies

The 19 SNPs located in the 14 candidate genes were successfully genotyped in the 122 backcrossed animals and showed minor allele frequencies (MAFs) ranging from 0.08 for *SERPINE1*:g.1249A>G to 0.46 for *COPA*:g.48255C>T. Most of the SNPs showed intermediate frequencies, and only four showed a MAF < 0.25 (Table 5). The two SNPs in the *JAK1* gene (g.18273A>G and g.12288A>G) appeared fully linked with a MAF = 0.38.

The association analysis results (Table 5) revealed coherent effects of the *RETSAT* polymorphisms (ss1985400207 and ss1985400205) on ham weight, although the association was not significant after multiple testing correction.

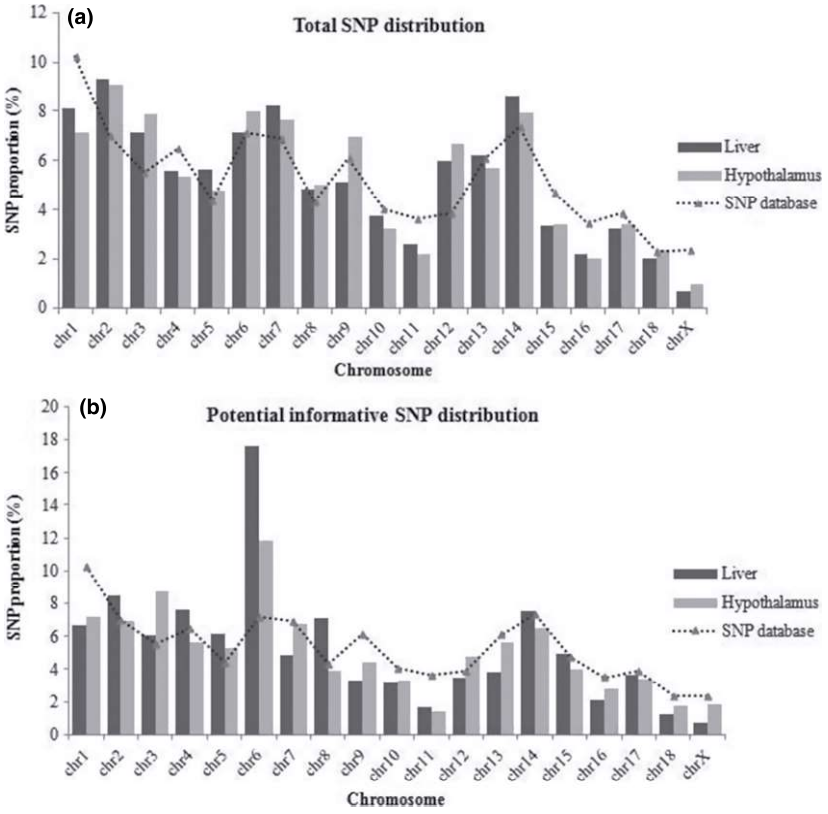

Figure 1 Distribution of the (a) total SNP and (b) potential informative SNP proportion detected in hepatic and hypothalamic tissues along porcine chromosomes.

Table 4 SNP set selected for validation by Sanger sequencing on cDNA samples.

SNP identification	Chromosome position	SNPdb no.	Gene name	Amino acid change	Validation by Sanger sequencing
ENSSSCG00000027057g.2895G>T	2:6195341	ss1985400201	*SYVN1*		Yes
ENSSSCG00000014401g.102797T>C	2:151055783	ss1985401074	*NR3C1*		Yes
ENSSSCG00000014411g.33280A>C	2:153858779	ss1985400202	*LARS*	Lys614Thr	Yes
ENSSSCG00000014411g.7010G>A	2:153885049	ss1985400203	*LARS*	Asp127Asn	Yes
ENSSSCG00000007858g.13374T>A	3:26152993	ss1985401075	*ACSM2B*	Ser272Thr	Yes
ENSSSCG00000008237g.3320A>C	3:62319781	ss1985400207	*RETSAT*	Lys173Thr	Yes
ENSSSCG00000006386g.48255C>T	4:98081626	ss1985400208	*COPA*		Yes
ENSSSCG00000006386g.48429G>T	4:98081800	ss1985401076	*COPA*		Yes
ENSSSCG00000006386g.48430G>T	4:98081801	ss1985401077	*COPA*		Yes
ENSSSCG00000006415g.21844T>C	4:99236075	ss1985401078	*CADM3*		Yes
ENSSSCG00000006530g.5633T>C	4:103449624	ss1985400210	*EFNA1*		Yes
ENSSSCG00000006582g.1877C>T	4:104969868	ss1985401079	*S100A14*		Yes
ENSSSCG00000006727g.49406C>G	4:112791607	ss1985401080	*WDR3*		Yes
ENSSSCG00000006874g.210G>C	4:129990914	ss1985400211	*PALMD*		Yes
ENSSSCG00000006887g.17250A>G	4:134252015	ss1985400212	*CNN3*		Yes
ENSSSCG00000000075g.10685T>C	5:5200373	ss1985400213	*MKL1*	Val245Ala	Yes
ENSSSCG00000028272g.48226C>T	5:16572785	ss1985401081	*FP565371.2*	Ala109Val	Yes
ENSSSCG00000030076g.27047A>G	5:69870959	ss1985401082	*SLC6A13*		No
ENSSSCG00000003247g.4656C>T	6:52789410	ss1985401083	Non-annotated		No
ENSSSCG00000003651g.19048A>G	6:87893864	ss1985400214	*RHBDL2*	Met19Val	Yes
ENSSSCG00000003809g.18273A>G	6:135899621	ss1985401084	*JAK1*		Yes
ENSSSCG00000003809g.12288A>G	6:135905604	ss1985400215	*JAK1*		Yes
ENSSSCG00000023489g.4832C>A	8:75801501	ss1985401085	*CXCL9*		No
ENSSSCG00000010884g.14128A>G	10:21630979	ss1985401086	Non-annotated		No[1]
ENSSSCG00000027815g.4525A>G	11:22178068	ss1985401087	*COG3*	Ile83Val	Yes
ENSSSCG00000017383g.4388C>A	12:20231782	ss1985401088	*AOC3*		No
ENSSSCG00000010487g.9565G>A	14:116122358	ss1985401089	Non-annotated		No
ENSSSCG00000010488g.39756G>A	14:116244344	ss1985401090	Non-annotated		No
ENSSSCG00000010739g.20840C>G	14:145893720	ss1985401091	*CTBP2*		Yes
ENSSSCG00000027439g.7569A>T	17:18863266	ss1985400216	*HAO1*		Yes
ENSSSCG00000025855g.185A>C	GL893425.2:41788	ss1985400217	*RNMT*	Glu62Ala	Yes
ENSSSCG00000025698g.1249A>G	GL894574.1:17487	ss1985400218	*SERPINE1*	Ile150Val	Yes

[1]No amplification.

Both *RETSAT*:g.3320C and g.3661delT alleles showed similar effects: an increase of around 200 g on mean ham weight. Haplotypes were built for these two polymorphisms (H1: g.3320C/g.3661insT; H2: g.3320C/g.3661delT; H3: g.3320A/g.3661insT), and the association analysis revealed more relevant additive effects than did the single analysis (Fig. 2). A comparison of the H1 (CT) and H2 (C–) haplotypes with H3, showed an increase in ham and bone-in-loin mean weights and leading to a potential reduction in backfat thickness. Effects on premium cut yields were also found for the *RNMT* polymorphism (ss1985400217): the *RNMT*:g.185C allele was associated with a 200 g increase in ham weight, a 100 g increase in shoulder weight and a 200 g increase in the bone-in-loin mean weight. Moreover, large effects were detected for the *COPA* and *PALMD* polymorphisms (ss1985400208 and ss1985400211) on backfat thickness at 75 kg (Table 5). The *COPA*:g.48255T allele was associated with an increase in fat deposition of about 0.5 mm, whereas the *PALMD*:*g.*210C allele was associated with an increase fat deposition of about 0.8 mm. No effect could be detected for body weight or intramuscular fat.

Additionally, differential gene expression conditional on genotypes for the significantly associated SNPs was tested on the available RNA-Seq data. Expression differences in liver were found for the *RETSAT* and *PALMD* transcripts conditional on the respective genotypes (Table 6). Moreover, relative gene expression was measured by qPCR on hepatic samples carrying different *RETSAT* genotypes: g.3320A>C, g.3453A>C, g.3661delinsT, g.3962A>G (ss1985400207, ss1985400206, ss1985400205 and ss1985400204 respectively). *RETSAT*:g.3962A>G showed significant expression differences: the g.3962G carriers expressed 0.147 times more than did the A carriers (*P*-value = 0.02). Moreover, *RETSAT* haplotypes were also built, and an association analysis with gene expression data was conducted. Four haplotypes were identified in the 31 analyzed samples, AATA (freq = 0.27), CATA (freq = 0.26), CA–T (freq = 0.15) and CCTG (freq = 0.32). The results allowed us to identify *RETSAT* expression differences between the CCTG and CATA haplotypes (fold change = 0.18, *P*-value = 0.01). However, *PALMD* gene expression differences conditional on its genotype could not be validated by qPCR in a larger sample set.

Table 5 Association analyses results of *SYVN1, LARS, RESTSAT, COPA, CADM3, EFNA1, PALMD, CNN3, MKL1, RHBDL2, JAK1, HAO1, RNMT* and *SERPINE1* polymorphisms on fatness (backfat thickness at 75 kg) and premium cut yield (ham, shoulder and bone-in-loin weights) related traits in the backcross (Iberian × Landrace) × Landrace.

			Additive effect on the trait (standard error)			
Polymorphism	SNPdb no.	MAF	BFT75 (mm)	HW (kg)	SW (kg)	BLW (kg)
*SYVN1:g.*2895G>T	ss1985400201	0.10	0.398 (0.329)	−0.160 (0.145)	−0.077 (0.079)	−0.164 (0.137)
*LARS:g.*33280A>C	ss1985400202	0.38	0.304 (0.201)	0.074 (0.094)	0.032 (0.051)	−0.013 (0.087)
*LARS:g.*7010G>A	ss1985400203	0.38	0.304 (0.199)	0.071 (0.093)	0.033 (0.051)	−0.010 (0.086)
*RETSAT:g.*3320A>C	ss1985400207	0.32	−0.298 (0.191)*	0.214 (0.087)*	0.032 (0.049)	0.149 (0.081)¥
*RETSAT:g.*3453A>C	ss1985400206	0.29	−0.322 (0.278)	−0.139 (0.132)	−0.017 (0.072)	0.011 (0.122)
*RETSAT:g.*3661delinsT	ss1985400205	0.14	0.042 (0.294)	0.281 (0.132)*	0.015 (0.073)	0.238 (0.121)¥
*RETSAT:g.*3962A>G	ss1985400204	0.45	−0.063 (0.259)	0.142 (0.119)	−0.015 (0.064)	0.118 (0.109)
*COPA:g.*48255 C>T	ss1985400208	0.46	0.528 (0.180)**€	−0.018 (0.086)	−0.060 (0.046)	−0.049 (0.078)
*CADM3:g.*21844T>C	ss1985401078	0.14	0.525 (0.282)¥	−0.131 (0.140)	−0.109 (0.074)	−0.075 (0.127)
*EFNA1:g.*5633T>C	ss1985400210	0.31	−0.318 (0.194)	0.021 (0.097)	0.046 (0.052)	−0.068 (0.087)
*PALMD:g.*210G>C	ss1985400211	0.31	−0.825 (0.200)***€	0.112 (0.098)	0.084 (0.052)	0.076 (0.092)
*CNN3:g.*17250A>G	ss1985400212	0.30	0.400 (0.205)¥	−0.037 (0.096)	−0.051 (0.052)	−0.052 (0.089)
*MKL1:g.*10685T>C	ss1985400213	0.40	0.034 (0.179)	−0.043 (0.082)	−0.056 (0.044)	−0.023 (0.076)
*RHBDL2:g.*19048A>G	ss1985400214	0.32	−0.187 (0.201)	0.167 (0.099)	0.090 (0.051)¥	0.164 (0.088)¥
*JAK1:g.*12288A>G	ss1985400215	0.38	0.265 (0.213)	−0.020 (0.103)	0.041 (0.055)	−0.089 (0.093)
*HAO1:g.*7569A>T	ss1985400216	0.34	0.156 (0.265)	0.052 (0.122)	0.045 (0.066)	−0.016 (0.112)
*RNMT:g.*185A>C	ss1985400217	0.34	−0.123 (0.220)	0.205 (0.099)*	0.104 (0.050)*	0.218 (0.087)*
*SERPINE1:g.*1249A>G	ss1985400218	0.08	−0.024 (0.354)	−0.172 (0.163)	−0.045 (0.084)	0.060 (0.147)

BFT75, body weight at 150 days (kg); HW, ham weight; SW, shoulder weight; BLW, bone-in-loin weight.
¥$P < 0.10$; *$P < 0.05$; **$P < 0.005$; ***$P < 0.0005$; €Significant after Bonferroni correction.

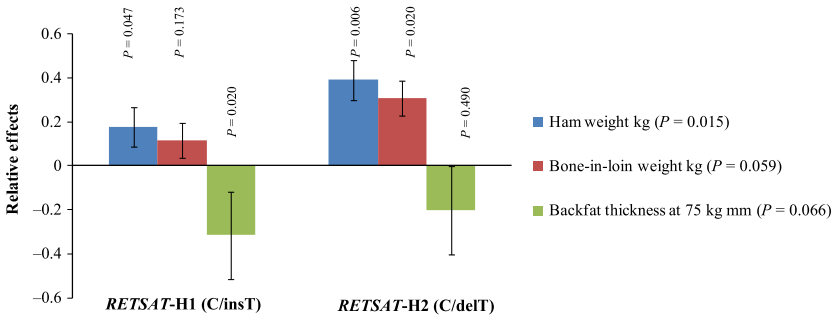

Figure 2 Graphical representation of the relative effects on ham weight, bone-in-loin and backfat thickness of *RETSAT* haplotypes H1 (g.3320C–g.3661insT) and H2 (g.3320C–g.3661delT) vs. H3 (*RETSAT:g.*3320A–g.3661insT).

RNA editing

Three of the validated SNPs on the *nuclear receptor subfamily 3 group C member 1* (*NR3C1*, *ENSSSCG00000014401:* g.102797T>C, ss1985401074), *component of oligomeric golgi complex 3* (*COG3*, *ENSSSCG00000027815*: g.4525A>G, ss1985401087) and *Acyl-CoA synthetase medium-chain family member 2B* (*ACSM2B*, *ENSSSCG00000007858*:g.13374T>A, ss1985401075) revealed different genotypes conditional on the type of analyzed tissue (Table 7), which might indicate tissue-specific allelic expression. The results of validation through Sanger sequencing on genomic DNA showed unexpected genotypes (Table 7 and Fig. 3). Some transcripts showed alleles that are not present in the corresponding gDNA sequence. These results were also validated by genotyping through the pyrosequencing method (Fig. 3). The results as a whole suggest the detection of RNA editing phenomena, i.e. RNA nucleotide substitutions (A>G, A>U, U>A), leading to differences between the final RNA sequence and the DNA region from which it was transcribed.

Moreover, genotyping by sequencing was conducted on an additional set of hypothalamic cDNA and gDNA samples from six animals (pure Iberian and Iberian × Large White). The sequencing of these additional animals with different genetic origins revealed the same genotype profiles as previously detected for the three SNPs (Table 7).

Discussion

SNP detection

In the current study, an effort was made to evaluate the usefulness of RNA-Seq technology to successfully identify valuable SNPs. Moreover, detection of powerful candidate

genes and mutations underlying production trait variations in the IBMAP population and RNA editing phenomena were detected and validated.

More than 125 000 SNVs were identified in each tissue, and 78% were considered potential SNPs. A SNV was considered as segregating if at least two animals were heterozygous or homozygous for the alternative allele within the analyzed samples. Thus, 22% of the identified SNVs could correspond either to fixed variants in the population studied (Iberian and Landrace vs. Duroc) or errors in the reference sequence (Duroc). Although the assay was focused on gene expression analysis, 35% of the identified SNPs mapped to within intronic regions, which probably points to the existence and sequencing of immature RNAs and a large number of non-annotated transcripts due to the incompleteness of the available porcine genome annotation. Among the SNPs falling within annotated exons, the ratio of synonymous to non-synonymous polymorphisms was 2.2:1 in hypothalamus and 2.4:1 in liver, showing as expected (Schattner & Diekhans 2006) that a large proportion of the identified SNPs do not lead to amino acid changes.

Further analyses were conducted to identify potentially informative SNPs in the H and L groups of the experimental backcross that could lead to causal genes and mutations for growth, fatness and meat yield. Because of the limited number of samples used in the RNA-Seq assay, a strict criterion, SNPs showing a homozygous or heterozygous genotype in one group and the alternative genotype in the other group, was set up. Due to the nature of the backcrossed population, we did not expect to find informative and causative mutations displaying alternative alleles between the established groups. As could be expected, the number of potentially informative SNPs was greatly reduced to 4396 in hypothalamus and 1862 in liver.

The reliability of the sequencing data, read filtering, mapping and SNP calling processes was evaluated using a set of 32 SNPs selected using standard Sanger sequencing. Only five false positive SNPs were detected. A deeper

Table 6 Gene expression differences conditional on polymorphism genotypes for *RETSAT, RNMT*, *COPA* and *PALMD* transcripts based on nine RNA-Seq measures.

Transcript	Liver RPKM (SD)	Hypothalamus) RPKM (SD
*RETSAT:*g.3320C>A; g.3661indelT (ss1985400207; ss1985400205)		
H3	85.37 (25.03)	23.08 (3.74)
H1/H2	30.17 (4.42)	27.44 (0.95)
Ratio	2.83	0.84
P-value	0.03	0.12
*RNMT:*g.185A>C (ss1985400217)		
AC	4.65 (0.27)	16.55 (1.40)
CC	4.50 (0.42)	13.58 (3.87)
Ratio	1.03	1.21
P-value	0.59	0.28
*COPA:*g.48255 C>T (ss1985400208)		
CC	31.29 (0.94)	49.29 (4.32)
TT	50.19 (10.36)	45.72 (4.04)
Ratio	0.62	1.08
P-value	0.12	0.35
*PALMD:*g.210G>C (ss1985400211)		
CC	10.76 (0.21)	6.39 (1.31)
GC	2.61(2.01)	5.55 (1.59)
Ratio	4.12	1.15
P-value	0.002	0.48

RPKM, reads per kilobase of transcript per million mapped.

Table 7 SNPs showing different genotype conditional on tissue type; validation by Sanger sequencing on cDNA from hypothalamic and hepatic tissues and on genomic DNA. Additional sample validation: Pure Iberian (IB) and Iberian × Large White (IB × LW).

	*COG3:*g.4525A>G (ss1985401087)			*ACSM2B:*g.13374A>T (ss1985401075)			*NR3C1:*g.102797T>C (ss1985401074)		
	Hypothalamus	Liver	gDNA	Hypothalamus	Liver	gDNA	Hypothalamus	Liver	gDNA
H-group									
Individual 1	AG	GG	AA	AT	AA	AA	CT	CT	TT
Individual 2	AG	GG	AA	AT	AA	AA	CC	CC	CC
Individual 3	AG	GG	AA	AA	AA	AA	CC	CC	CC
Individual 4	AG	GG	AA	AT	AA	AA	CC	CC	CC
L-group									
Individual 5	AG	GG	AA	AT	TT	AA	CT	CT	TT
Individual 6	AG	GG	AA	AT	AT	AA	CC	CC	CC
Individual 7	AG	GG	AA	AA	AT	AA	CC	CC	CC
Individual 8	AG	GG	AA	AA	AT	AA	CC	CT	TT
Individual 9	AG	GG	AA	AA	AT	AA	CT	CT	TT
Additional sample set									
IB1	AG	–	AA	AT	–	AA	CC	–	CC
IB2	AG	–	AA	AA	–	AA	CT	–	TT
IB3	AG	–	AA	AT	–	AA	CC	–	CC
IB × LW1	AG	–	AA	AA	–	AA	CC	–	CC
IB × LW2	AG	–	AA	AA	–	AA	CC	–	CC
IB × LW3	AG	–	AA	AT	–	AT	CC	–	CC

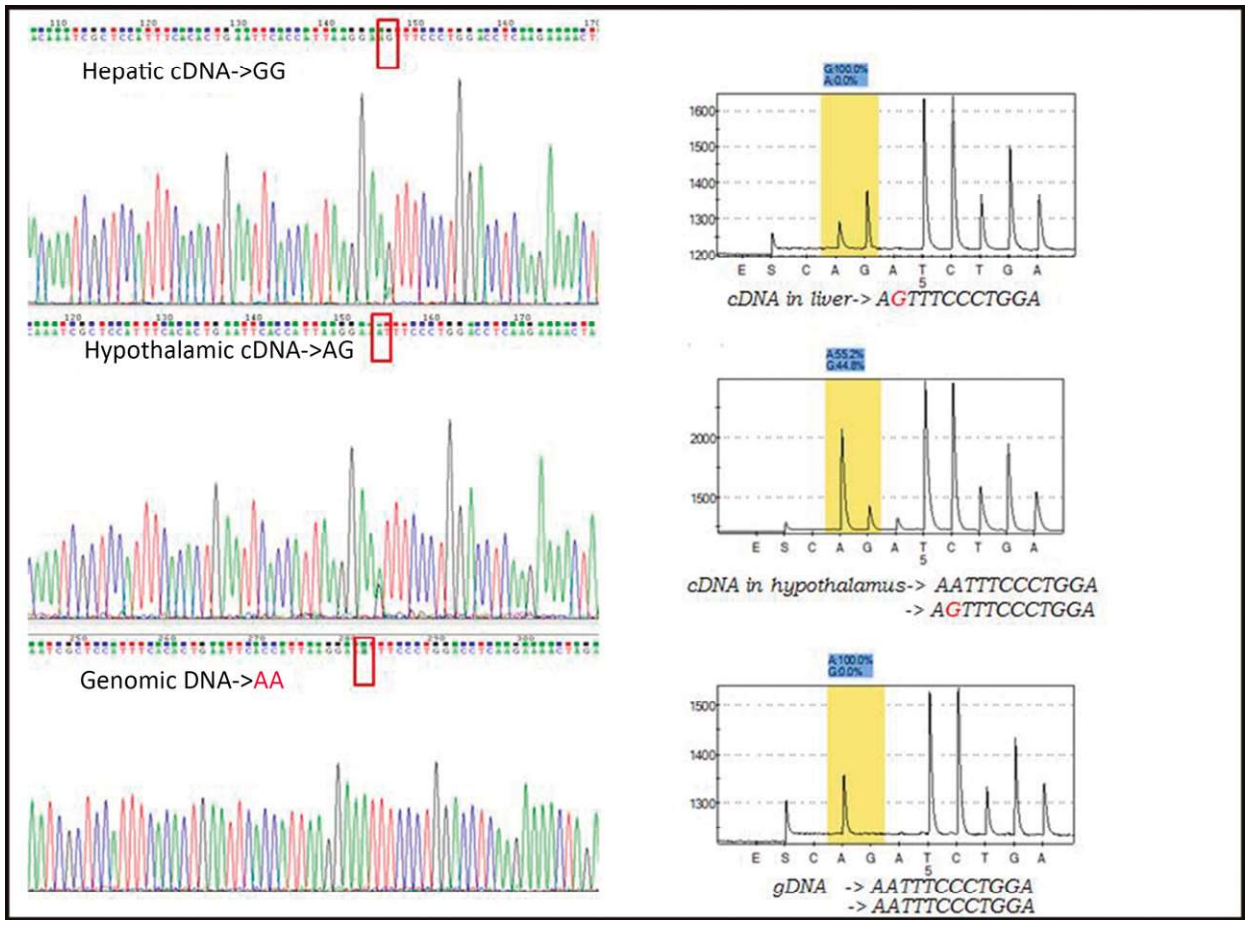

Figure 3 *ENSSSCG00000027815:g.*4525A>G SNP (ss1985401087) validation by Sanger sequencing and pyrosequencing on cDNA from hypothalamus and liver and on genomic DNA.

examination of the sequences covering these false positive SNPs allowed us to determine that the main factor influencing this result was likely mapping errors due to the excess of coverage in highly expressed transcripts. The SNP calling from RNA-Seq data, in contrast with whole genome sequence analysis, has to allow for high coverage because it is conditional on gene expression level, which may increase the chance of sequence errors at these highly sequenced genes. Here, the sequencing errors caused by the sequencing technology should also be taken into account, as Illumina technology shows error rates ranging from 0.3% at the beginning of reads to 3.8% at the end of reads (Dohm *et al.* 2008). Therefore, a more restricted criterion in the SNPs surrounding bases (i.e. from 5 to 10 bp) and an increase in the minimum read per allele (i.e. from 20% to 30%) could reduce the FDR.

The SNP proportions per chromosome identified here do not differ from those of the Ensembl porcine SNP database; however, when focusing on potential informative SNPs, the proportions do differ, especially for SSC6. SSC6 is particularly relevant in the IBMAP population, as a major QTL for growth, fat deposition and conformation has been mapped there (Óvilo *et al.* 2000; Varona *et al.* 2002), therefore a large degree of polymorphism is expected in this region between the parental breeds (Iberian and Landrace). Moreover, the *LEPR* gene is the main candidate for this QTL, as previously reported in several studies (Óvilo *et al.* 2000, 2002, 2010; Galve *et al.* 2012), which seems to have a specific behavior and sequence variation in Iberian pigs that is not found in other porcine breeds (Torres-Rovira *et al.* 2012; Pérez-Montarelo *et al.* 2015).

Association analyses

Genotyping and association analyses in the backcrossed population were conducted for 19 of the validated SNPs. The frequencies obtained indicate that the selection was successful for the identification of potential informative SNPs, as most SNPs showed intermediate frequencies in the population (MAF > 0.25), which are optimal in association analyses (Tabangin *et al.* 2009). Here, the limitation in the

population size, which makes it difficult to estimate highly significant allelic or genotype effects, should be taken into account. However, the association analyses revealed interesting results: The *RETSAT* and *RNMT* polymorphisms showed effects on premium cut yields, and the *COPA* and *PALMD* polymorphisms showed significant effects on backfat thickness.

Functional candidate polymorphisms

The *RETSAT* polymorphisms were selected for validation and posterior association analysis due to the biological implication of the RetSat enzyme in retinoid metabolism. RetSat is induced during adipogenesis and is directly regulated by the transcription factor peroxisome proliferator activated receptor alpha and gamma in adipose and hepatic tissues respectively. Moreover, RetSat promotes adipogenesis (Schupp *et al.* 2009) and lipid accumulation (Moise *et al.* 2010). Our results agree with the role attributed to RetSat. The *RETSAT*:g.3320A>C and *RETSAT*:g.3661delinsT polymorphisms show opposite effects on cut yields and backfat thickness, as expected given that the RetSat enzyme promotes fat deposition. Moreover, the haplotype analysis confirms these effects. The H1 (CinsT) and H2 (CdelT) haplotypes are associated with an increase in premium cut yields and a reduction in backfat thickness. On the other hand, H3 (AinsT) is associated with increases in fat deposition and decreases in premium cut yields. The potential effects of these polymorphisms on mRNA, and protein structure were evaluated using *in silico* tools. The most interesting corresponded to the *RETSAT*:g.3320A>C polymorphism, leading to p.Lys173Thr, which is predicted to be tolerated by SNAP2 and SIFT tools. However, this polymorphism matched an exon enhancer (REGRNA prediction tool), whose pattern is GAAGAA (Liu *et al.* 2003). The SNP would change this pattern to GAAGAC, suppressing the enhancer. *RETSAT*:g.3661delinsT corresponds to an intronic indel, which could constitute a target site for hsa-miR-1266 (miRNA: 3′-ucGGGACAAGAUGUCGGGACUCc-5′ → target: 5′-acCCTTTTTCCCTTTCTCTGAGa-3′) following the REGRNA prediction tool. The indel alters the micro-RNA target to 5′-acCCTTTTCCCTTTCTCTGAGa-3′. Although these results are based on predictions, they indicate that these mutations could affect gene expression regulation. However, our gene expression result revealed significant gene expression differences only for the CATA vs. CCTG *RETSAT* haplotypes and therefore do not fully fit with the obtained phenotypic association results, which may indicate that the analyzed polymorphisms show different levels of linkage disequilibrium with the actual causative mutation.

The *RNMT*:g.185A>C polymorphism revealed effects on the weights of ham, shoulder and bone-in-loin premium cut yields. The RNMT enzyme is required for cell proliferation processes (Aregger & Cowling 2013) implicated in muscle growth, and consequently, it influences premium cut yields. The evaluated polymorphism leads to p.Gln62Lys, also predicted to be tolerated by SNAP2 and SIFT. The REGRNA prediction tool shows a change in the RNA exposition and accessibility due to the nucleotide substitution (Fig. 4), which could affect post-transcription and protein expression. RNA-Seq gene expression data did not reveal gene expression differences conditional on genotype from liver or hypothalamus, which could support the hypothesis of post-transcriptional effects.

Positional candidate polymorphisms

The *COPA*:g.48255C>T and *PALMD*:g.210G>C polymorphisms showed effects on backfat thickness. These genes were selected for further analyses because they both map within QTL regions of SSC4 for backfat thickness (Fernández *et al.* 2012). Fernández *et al.* (2012) identified two QTL on SSC4, one at the well-known *FAT1* (Marklund *et al.* 1999; Silva *et al.* 2011) at 72–91 cM (97–117 Mb) and a second one at 102–109 cM (129–134 Mb) positions. The *COPA* and *PALM* genes exactly match those two QTL intervals, at 98 Mb at 129 Mb respectively. The association found for the *COPA* gene could be a consequence of the linkage disequilibrium with other powerful candidates genes located in this region, such as *FABP4* and *FABP5*, and analyzed in previous studies (Estellé *et al.* 2006). Validation analysis would be required in order to determine which of these genes (*FABP4*, *FABP5* or *COPA*) is the actual gene carrying the causal mutation for *FAT1*.

Despite *PALMD*:g.210G>C being a synonymous SNP, it could impact gene expression, as shown for the hepatic RNA-Seq gene expression (Table 6). The *in silico* analysis performed with the REGRNA tool predicted that this mutation could fall within a transcriptional regulatory motif (Accession no. R0147; Maekawa *et al.* 1989) in exon 1. The mutation would change the pattern from AGCGGA/TCCGCT to AGGGGA/TCCCCT, affecting gene expression. However, we could not validate the differences in gene expression conditional on the *PALMD* genotype in a larger sample set. It should be noted that, because the tested *PALMD* SNP was in a known QTL region (therefore in linkage disequilibrium with the QTL), it is difficult to know the true polymorphism effect and if it actually corresponds to a causal mutation.

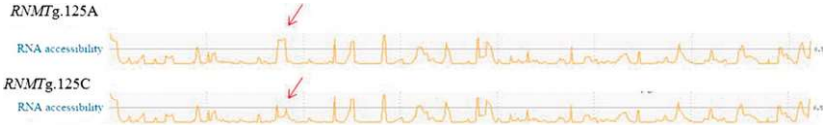

Figure 4 REGRNA prediction of effect on RNA accessibility for the *RNMT*:g.185A>C polymorphism (ss1985400217).

Although further functional and genetic validations are needed to prove the association of the polymorphisms identified here, the *RETSAT*, *RNMT* and *PALMD* genes constitute powerful candidates to influence fat deposition and premium cut yields.

RNA editing

Interestingly, RNA editing was detected in the current study when RNA-Seq SNP validation was conducted. The *NR3C1*: g.4525A>G, *COG3*:g.13374T>A and *ACSM2B*:g.13374T>A RNA editing modifications have been validated. Moreover, the same patterns were identified when genotyping was performed in other genetic backgrounds (pure Iberian and Iberian × Large White), indicating that it is a common mechanism.

The most common types of RNA editing modifications in vertebrates are the A-to-I conversion, leading to an A-to-G reading of the cDNA molecule and catalyzed by an adenosine desaminase that acts on RNA family enzymes (ADAR) and the C-to-U conversion, catalyzed by the APOBEC enzyme (Bass 2002; Blanc & Davidson 2003). In the current study the modifications detected were one of the most common conversions: A-to-G (A-to-I in mRNA) or rather A-to-T (A-to-U in mRNA) and T-to-C (U-to-A in mRNA). Functional implications of these modifications were further analyzed by *in silico* sequence analyses.

The missense modification of *COG3*:g.4525A>G has been previously described in human, mouse and rat (Shah *et al.* 2009; Danecek *et al.* 2012; Holmes *et al.* 2013) and more recently in chickens (Frésard *et al.* 2015), proving that at least some RNA editing conservation occurs across vertebrate species. The modification on the component of oligomeric Golgi complex 3 (COG3), a key molecule in protein metabolism, is missense, a p.Ile83Val change, which is functional but tolerated following SNAP2 and SIFT prediction tools. The REGRNA sequence analysis revealed that the modification lies in an exon enhancer region (Accession no. R0815), motif GGAAG, involved in the promotion of alternative splicing (Dirksen *et al.* 2003), which is likely linked to different biological functions conditional on tissue type, as evidenced in the current results showing different transcripts conditional on tissue type.

The *NR3C1:*g.102797T>C and *ACSM2B:*g.13374A>T modifications have not been previously described. The modification in *NR3C1*, involved in glucocorticoid resistance, lies in the 3′ untranslated region. The REGRNA prediction tool shows that the *NR3C1*g.4525 modification could lie in a potential target site for the hsa-miR-2054 miRNA (miRNA: 3′-uuaUUUAAUUUAAAU-------AUAAUGUc-5′ → target: 5′-agaAAGTTGAATTTATAGCTTTTATTGTAc-3′). Finally, the modification on *ACSM2B* produces p.Ser272Thr. This change is predicted by the SIFT tool to be tolerated, but REGRNA predicts a splicing site implicating the *ACSM2B*:g.13374 position. Moreover, this modification can be considered of major interest because the two different hepatic transcripts showed variations conditional on the tissue that could be functionally related to the H and L animal groups used for the RNA-Seq assay.

The current study has revealed the relevance of the RNA editing, its existence and potential impact, and highlights that RNA editing should be taken into account for future genetic analysis. Furthermore, it could impact the estimates of false discovery rates in SNP validation from RNA-Seq assays due to the differences between the RNA sequence and the DNA region from which it was transcribed.

Here, we have provided valuable SNP data, from over 90 000 SNPs, useful for future studies. Additionally, further approaches can be proposed to validate the promising results obtained, including validations of the polymorphism effects on fatness and premium cut yields and studying the putative functionality of the detected RNA editing modifications.

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Supporting information

Additional supporting information may be found online in the supporting information tab for this article:

Table S1. Primers and amplification conditions used for SNP validation, Sanger sequencing on cDNA and gDNA, pirosequecing and qPCR.

Artículo II

Deciphering the regulation of porcine genes influencing growth, fatness and yield-related traits through genetical genomics

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Deciphering the regulation of porcine genes influencing growth, fatness and yield-related traits through genetical genomics

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Abstract Genetical genomics approaches aim at identifying quantitative trait loci for molecular traits, also known as intermediate phenotypes, such as gene expression, that could link variation in genetic information to physiological traits. In the current study, an expression GWAS has been carried out on an experimental Iberian×Landrace backcross in order to identify the genomic regions regulating the gene expression of those genes whose expression is correlated with growth, fat deposition, and premium cut yield measures in pig. The analyses were conducted exploiting Porcine 60K SNP BeadChip genotypes and Porcine Expression Microarray data hybridized on mRNA from *Longissimus dorsi* muscle. In order to focus the analysis on productive traits and reduce the number of analyses, only those probesets whose expression showed significant correlation with at least one of the seven phenotypes of interest were selected for the eGWAS. A total of 63 eQTL regions were identified with effects on 36 different transcripts. Those eQTLs overlapping with phenotypic QTLs on SSC4, SSC9, SSC13, and SSC17 chromosomes previously detected in the same animal material were further analyzed. Moreover, candidate genes and SNPs were analyzed. Among the most promising results, a long non-coding RNA, *ALDBSSCG0000001928*, was identified, whose expression is correlated with premium cut yield. Association analysis and in silico sequence domain annotation support *TXNRD3* polymorphisms as candidate to regulate *ALDBSSCG0000001928* expression, which can be involved in the transcriptional regulation of surrounding genes, affecting productive and meat quality traits.

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Introduction

Several approaches are now available in order to elucidate genetic architecture of complex traits such as growth, fat deposition, carcass composition, or meat quality in livestock species. Traditionally phenotypic QTL (QTL) mapping has been carried out using linkage analysis with limited number of microsatellite markers (Wang et al. 2002; Deng et al. 2000). Although this approach provided reliable results, further analyses to identify underlying genes or causative mutations have not been very successful, in part due to the lack of QTL position precision limited by the available markers (Würschum and Kraft 2014; Schön et al. 2004). More recently, Genome-Wide Association Study (GWAS) using high density SNP panels has emerged as a strong approach that minimizes the marker number limitation (Hill 2012; Sun et al. 2015). GWAS analyses are usually focused on the study of phenotypic traits using genomic data. Nevertheless, it is not the only possible application; genetical genomics studies (Jansen and Nap

2001; Breitling et al. 2008) can be conducted by carrying out expression GWAS (eGWAS). Genetical genomics aims at identifying QTL for molecular traits, also known as intermediate phenotypes, such as gene expression (eQTL) that could link variation in genetic information to physiological traits (Williams et al. 2007). These analyses allow us to obtain information regarding gene expression regulation, regulation paths, and interactions that could help understand the genetic architecture of complex traits (Zou et al. 2012). Studies in human have validated the successfulness of eGWAS to identify variants associated with complex human diseases, and the potential role of gene expression changes in those diseases (Kodama et al. 2012; Zou et al. 2012).

Previous studies in an Iberian×Landrace porcine experimental cross using both linkage and GWAS analyses allowed us to identify QTL with relevant effects on growth, fat deposition, and premium cut yield-related traits (Óvilo et al. 2000; Varona et al. 2002; Mercadé et al. 2005; Fernández et al. 2012, 2014). However, the identification of potential causal variants has been limited to *ELOVL6, LEPR*, and *FABP* genes (Corominas et al. 2013, 2015; Ovilo et al. 2005; Estellé et al. 2006; Pérez-Montarelo et al. 2012). Therefore, the aim of the current study is to help in the identification of potential causal variants for these traits through a genetical genomics study. An eGWAS study has been carried out on an experimental Iberian×Landrace backcross in order to identify the genomic regions regulating the gene expression of those genes whose expression is correlated with growth, fat deposition, and premium cut yield measures. The analyses were conducted exploiting Porcine 60K SNP BeadChip genotypes and Porcine Expression Microarray (Affymetrix) data hybridized on mRNA from *Longissimus dorsi* muscle.

Materials and methods

Animals

The phenotypic information and gene expression data used in the current study belong to an experimental backcross F1 (Iberian×Landrace)×Landrace of the IBMAP population (Óvilo et al. 2000; Mercadé et al. 2005; Óvilo et al. 2005). The IBMAP F1 generation was obtained from three Iberian Guadyerbas boars and 30 Landrace sows, five of these F1 boars were mated with 25 Landrace sows obtaining 160 animals from the backcross (BC).

All animal procedures were performed according to the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 86/609 about the protection of animals used in experimentation.

Phenotypic data

For the study seven traits related to growth, fatness, and meat quality were recorded (Table 1). These were body weight at 150 days of mean age (BW150), backfat thickness measured at 75 kg (BFT75) and at slaughter (BFS), weights of premium cuts, such as hams (HW), shoulders (SW), and loin bone-in (LBW), and intramuscular fat content (IMF) measured in *Longissimus dorsi* samples at slaughter (Fernandez et al. 2012).

Gene expression

Gene expression data were obtained from the hybridization of mRNA samples coming from *Longissimus dorsi* of 102 backcrossed individuals with the Porcine Expression Microarray (Affymetrix) as described in Pena et al. (2013). Quality control was carried out with the microarray data using affyPLM package of the Bioconductor software (http://www.bioconductor.org/). RNA normalization was carried using BRB-Array Tools (v. 3.6.0) (http://linus.nci.nih.gov/BRB-ArrayTools.html). Expression data are expressed as the log2 of probeset signal intensity.

Correlation (phenotype and expression)

A correlation analysis was carried out between phenotypic (BW150, BFT75, BFS, HW, SW, LBW, and IMF) and expression data. Expression and phenotypic data were corrected adjusting a linear model, setting sex and batch as fixed effects, and slaughter age as random effect. Pearson correlation coefficient was calculated between the predicted values from 24,000 probesets and the predicted values of the seven phenotypic records. Genes with significant correlation levels ($r = |0.32| - |0.66|$, p value <0.001, q value <0.002) were selected for further analysis. Microarray probesets were annotated using NetAff from Affymetrix (https://www.affymetrix.com/analysis/index.affx).

Genotyping data

DNA samples from 160 backcrossed and their F1 and F0 relatives were genotyped with the PorcineSNP60 BeadChip (Illumina, Inc.), designed by Ramos et al. (2009). GenomeStudio software (Illumina, Inc.) was employed to visualize, edit, standardize quality filter, and extract genotyping data. A second process of data filtering was carried out with GenABEL software, those markers with a minimum allele frequency (MAF) <2.5%, and markers deviating from Hardy–Weinberg equilibrium (FDR < 1%) were discarded. A total of 31,606 SNPs were considered for further analyses.

Table 1 eQTL identified in the eGWAS analyses conducted *on Longissimus dorsi* gene expression data

eQTL						Associated gene expression					
Name	Chr	Start	End	Size (bp)	Num_SNPs	Probeset	Name	Chr	Gene start	Gene end	*Cis_Trans*
R1	1	8,988,180	15,231,294	6,243,114	11	*Ssc.1860.1.S1_at*	*DYNLT1*	1	10,385,075	10,391,604	*cis*
R2	1	129,136,194	130,167,976	1,031,782	8	*Ssc.25573.1.S1_at*	*HIVEP2*	1	24,926,110	24,957,597	*trans*
R3	1	146,180,618	146,332,008	151,390	5	*Ssc.20685.1.S1_at*	*BCL2*	1	175,695,537	175,696,139	*trans*
R4	1	309,039,258	309,125,803	86,545	2	*Ssc.30633.1.S1_at*	*MNS1*	1	127,767,312	127,821,308	*trans*
R5	2	16,416	10,979,357	10,962,941	28	*Ssc.9365.2.S1_a_at*	*IGF2*	GL896425.1	1	2,416	*trans*
R6	2	251,447	10,979,357	10,727,910	22	*Ssc.20525.1.S1_at*	*P09565*	GL896425.1	1	2,416	*trans*
R7	2	118,359,766	120,243,915	1,884,149	9	*Ssc.10952.1.S1_at*	*DLG1*	13	142,336,267	142,629,535	*trans*
R8	2	162,084,552	162,298,086	213,534	2	*Ssc.20525.1.S1_at*	*P09565*	GL896425.1	1	2,416	*trans*
R9	2	162,084,552	162,298,086	213,534	2	*Ssc.9365.2.S1_a_at*	*IGF2*	GL896425.1	1	2,416	*trans*
R10	3	14,221,604	14,772,381	550,777	2	*Ssc.29388.1.A1_at*	*SEMA3A*	JH118932.1	48,007	86,320	*trans*
R11	3	58,059,120	62,507,762	4,448,642	19	*Ssc.11208.1.S1_at*	*IGK*	3	59,720,209	59,881,293	*cis*
R12	3	93,221,408	97,347,171	4,125,763	8	*Ssc.7060.2.S1_at*	*FBXO11*	3	98,538,854	98,709,802	*trans*
R13	3	111,541,225	112,768,775	1,227,550	11	*Ssc.26316.1.S1_at*	*KTN1*	1	205,432,796	205,534,850	*trans*
R14	4	13,084,536	13,330,048	245,512	2	*Ssc.25555.1.S1_at*	*TATDN1*	4	15,769,901	15,816,051	*trans*
R15	4	15,848,929	16,347,684	498,755	4	*Ssc.25555.1.S1_at*	*TATDN1*	4	15,769,901	15,816,051	*cis*
R16	4	19,818,145	22,561,896	2,743,751	13	*Ssc.25555.1.S1_at*	*TATDN1*	4	15,769,901	15,816,051	*trans*
R17	4	28,899,542	29,558,810	659,268	2	*Ssc.25555.1.S1_at*	*TATDN1*	4	15,769,901	15,816,051	*trans*
R18	4	63,841,367	63,988,841	147,474	2	*Ssc.9109.1.A1_at*	*IDE*	14	113,505,019	113,583,601	*trans*
R19	4	128,983,125	129,016,956	33,831	2	*Ssc.7190.1.S1_at*	*BUB1B*	1	146,310,747	146,312,591	*trans*
R20	5	876,762	1,864,156	987,394	9	*Ssc.1790.1.S1_at*	*NUP50*	5	1,512,925	1,526,924	*cis*
R21	6	48,585,961	52,336,598	3,750,637	30	*Ssc.18795.1.A1_at*	*CNTN6*	1	63,899,864	63,922,174	*trans*
R22	6	50,478,565	50,888,554	409,989	3	*Ssc.894.1.A1_at*	*Mico1*	6	50,803,937	50,804,291	*cis*
R23	6	51,775,907	52,993,696	1,217,789	6	*Ssc.30633.1.S1_at*	*MNS1*	1	127,767,312	127,821,308	*trans*
R24	6	80,520,240	84,333,986	3,813,746	28	*Ssc.2152.1.A1_at*	*BSDC1*	6	82,782,946	82,808,253	*cis*
R25	6	85,813,657	86,899,252	1,085,595	3	*Ssc.11158.2.S1_at*	*PTP4A2*	6	82,337,830	82,348,373	*trans*
R26	6	155,897,700	157,126,627	1,228,927	10	*Ssc.21969.1.A1_at*	*C1orf50*	6	156,177,269	156,183,027	*cis*
R27	7	38,189,183	39,842,612	1,653,429	2	*Ssc.19634.1.S1_at*	*MRPL14*	7	44,942,222	44,955,752	*trans*
R28	7	44,767,409	48,179,859	3,412,450	9	*Ssc.19634.1.S1_at*	*MRPL14*	7	44,942,222	44,955,752	*cis*
R29	7	50,750,507	50,781,071	30,564	2	*Ssc.19634.1.S1_at*	*MRPL14*	7	44,942,222	44,955,752	*trans*
R30	7	128,891,964	131,361,899	2,469,935	24	*Ssc.15678.1.A1_s_at*	*CHCHD6*	GL894452.1	10,304	10,550	*trans*
R31	7	128,891,964	131,323,540	2,431,576	10	*Ssc.21543.1.S1_at*	–	–	–	–	*trans*
R32	8	8,934,560	11,962,779	3,028,219	29	*Ssc.10298.1.A1_at*	*TAPT1*	8	11,018,821	11,067,248	*cis*
R33	8	12,476,033	12,483,876	7,843	2	*Ssc.7713.1.A1_at*	*LAP3*	6	40,305,180	40,306,031	*trans*
R34	8	33,235,728	33,365,922	130,194	2	*Ssc.29017.1.A1_at*	*SETD6*	6	18,327,600	18,332,824	*trans*
R35	9	118,021,130	120,667,155	2,646,025	5	*Ssc.7190.1.S1_at*	*BUB1B*	1	146,310,747	146,312,591	*trans*
R36	11	3,914,900	23,463,154	19,548,254	24	*Ssc.9916.1.S1_at*	*SPG20*	11	12,231,552	12,255,683	*cis*

Table 1 (continued)

eQTL						Associated gene expression					
Name	Chr	Start	End	Size (bp)	Num_SNPs	Probeset	Name	Chr	Gene start	Gene end	*Cis_Trans*
R37	13	19,683,797	19,750,520	66,723	2	*Ssc.10589.1.A1_at*	*Ssc.10589.1.A1_at*	13	80,736,233	80,736,662	*trans*
R38	13	19,774,122	19,859,830	85,708	2	*Ssc.24608.1.S1_at*	*DAG1*	JH118489.1	81,292	97,231	*trans*
R39	13	25,730,204	31,650,967	5,920,763	5	*Ssc.10589.1.A1_at*	*Ssc.10589.1.A1_at*	13	80,736,233	80,736,662	*trans*
R40	13	26,286,725	27,911,595	1,624,870	8	*Ssc.24997.1.S1_at*	*LSAMP*	13	153,563,024	153,783,293	*trans*
R41	13	34,526,276	36,964,009	2,437,733	6	*Ssc.10589.1.A1_at*	*Ssc.10589.1.A1_at*	13	80,736,233	80,736,662	*trans*
R42	13	46,063,345	53,877,058	7,813,713	11	*Ssc.10589.1.A1_at*	*Ssc.10589.1.A1_at*	13	80,736,233	80,736,662	*trans*
R43	13	59,740,715	73,787,637	14,046,922	56	*Ssc.10589.1.A1_at*	*Ssc.10589.1.A1_at*	13	80,736,233	80,736,662	*trans*
R44	13	78,219,919	84,277,803	6,057,884	15	*Ssc.10589.1.A1_at*	*Ssc.10589.1.A1_at*	13	80,736,233	80,736,662	*cis*
R45	13	87,702,788	89,919,771	2,216,983	10	*Ssc.10589.1.A1_at*	*Ssc.10589.1.A1_at*	13	80,736,233	80,736,662	*trans*
R46	13	98,646,754	103,194,490	4,547,736	6	*Ssc.10589.1.A1_at*	*Ssc.10589.1.A1_at*	13	80,736,233	80,736,662	*trans*
R47	13	124,746,808	126,830,429	2,083,621	5	*Ssc.10589.1.A1_at*	*Ssc.10589.1.A1_at*	13	80,736,233	80,736,662	*trans*
R48	13	134,933,329	140,308,510	5,375,181	18	*Ssc.24542.1.S1_at*	*RGMB*	2	108,921,274	108,921,825	*trans*
R49	13	154,043,749	154,103,781	60,032	2	*Ssc.10589.1.A1_at*	*Ssc.10589.1.A1_at*	13	80,736,233	80,736,662	*trans*
R50	14	31,671,713	34,956,919	3,285,206	27	*Ssc.21861.1.S1_at*	*PYCRL*	4	1,013,502	1,013,974	*trans*
R51	14	100,004,352	120,439,354	20,435,002	209	*Ssc.9109.1.A1_at*	*IDE*	14	113,505,019	113,583,601	*cis*
R52	14	135,271,348	138,328,275	3,056,927	10	*Ssc.30987.1.S1_at*	*NHLRC2*	14	135,285,101	135,358,491	*cis*
R53	15	12,377,279	15,286,888	2,909,609	12	*Ssc.3249.1.S1_at*	*QSOX1*	9	133,595,707	133,637,533	*trans*
R54	15	99,540,054	101,622,603	2,082,549	14	*Ssc.16050.1.A1_at*	*ADAM17*	3	135,176,525	135,222,600	*trans*
R55	15	99,540,054	101,622,603	2,082,549	14	*Ssc.18795.1.A1_at*	*CNTN6*	13	63,839,664	63,863,130	*trans*
R56	17	18,894,036	25,670,863	6,776,827	15	*Ssc.7666.1.A1_at*	*PSMF1*	17	38,667,378	38,711,350	*trans*
R57	17	23,265,918	29,684,825	6,418,907	4	*Ssc.21242.1.S1_at*	*CTNNBL1*	17	46,357,154	46,401,936	*trans*
R58	17	28,475,777	29,684,825	1,209,048	2	*Ssc.7666.1.A1_at*	*PSMF1*	17	38,667,378	38,711,350	*trans*
R59	17	33,892,898	34,888,048	995,150	3	*Ssc.7666.1.A1_at*	*PSMF1*	17	38,667,378	38,711,350	*trans*
R60	17	38,047,955	41,558,311	3,510,356	7	*Ssc.7666.1.A1_at*	*PSMF1*	17	38,667,378	38,711,350	*cis*
R61	17	40,556,315	41,558,311	1,001,996	2	*Ssc.21242.1.S1_at*	*CTNNBL1*	17	46,357,154	46,401,936	*trans*
R62	17	45,164,920	54,774,793	9,609,873	16	*Ssc.21242.1.S1_at*	*CTNNBL1*	17	46,357,154	46,401,936	*cis*
R63	17	47,981,889	56,096,189	8,114,300	14	*Ssc.7666.1.A1_at*	*PSMF1*	17	38,667,378	38,711,350	*trans*

Description of the eQTL regions, including start and end positions, size, SNP number, affected probeset/gene, and *cis_trans* classification

eGWAS analysis

A genome-wide association study was performed using the GenABEL package (Karssen et al. 2016) in R environment. The analysis was carried on 102 individuals, those with expression and genotyping data. The genome-wide analysis was performed following the model:

$$y_{ijk} = S_i + B_j + bx_k + \sum_l \lambda_{lk} a_l + u_k + e_{ijk},$$

where y_{ijk} is the trait value of kth individual, S_i and B_j are fixed effects for sex and batch respectively, and b is the slaughter age regression coefficient. Additive effect of the SNP is a_l and λ_{lk} is the indicator related with the number of copies of the lth allele (0, 1, or 2) and u_k would be the infinitesimal effect of the kth individual, e_{ijk} is the random residual term. The same model but using carcass weight as regression coefficient showed similar results. QValue package in R was used to perform correction for multiple tests (Bass et al. 2015). Significant associations were considered for those reporting q value <0.05.

Region analysis

eQTLs were determined by two or more significantly associated SNPs within a maximum distance of 2 Mb. The genetic content of the eQTL was extracted using BioMart tool from Porcine Ensembl database. FatiGO and ReviGO online tools were used to investigate Gene Ontology enrichment and function. In order to prioritize the investigation on eQTL regions, those regions were compared with QTL regions obtained in previous studies carried out in the same material.

Candidate SNPs analyses

A candidate SNP search was done by exploiting an RNA-seq assay previously conducted on the same animal material (Martínez-Montes et al. 2016). The candidate SNPs were validated by Sanger sequencing on cDNA synthesized from mRNA. Primer pairs were designed from exon to exon, in order to avoid genomic DNA amplification (Supplemental Table 1). The PCR reactions were performed in a final volume of 25 µl, containing 4 µl of cDNA, 0.5 µl of polymerase, 2.5 µl buffer 10×, 2.5 µl of dNTPs, and 0.5 µl of each primer. Thermocycling was carried out under the following conditions: 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, with a final extension of 72 °C for 10 min. The PCR reactions were carried out in a GeneAmp PCR System 9700 (Applied Biosystems, Warrington, UK). The PCR products were purified with the illustra™GFX™ PCR DNA purification kit (GE Healthcare, UK) according to the manufacturers' protocol. PCR products were sequenced with both forward and reverse primers using the 3100 BigDye® Terminator v3.1 Matrix Standard in a 3730 DNA Analyzer (Applied Biosystems Warrington, UK). After validation, the SNPs were genotyped in the 160 backcrossed animals using different techniques: pyrosequencing using specific primers (Sup. Table 1); PCR-RFLP with restriction enzymes *Tsp451* (*ss2031475817*, GenBank ID: 100518810) and *BstUI* (*ss2031475807*, GenBank ID: 100233193); and the OpenArray platform at Servei Veterinari de Genètica Molecular (Universidad Autónoma de Barcelona, Spain).

The specific association analyses for the candidate SNPs and haplotypes, built with Phase 2.1.1 (Stephens et al. 2001), with gene expression measures were carried out using the previous quoted model using Qxpak software (Pérez-Enciso and Misztal 2011). Moreover, associations were also conducted for phenotypic traits. Bonferroni correction was applied to take into account multiple tests, setting up a p value of 0.003.

To examine the interest of the identified candidate SNPs, we analyzed in silico the potential effect of those SNPs that produce amino acid change using Predict Protein tool (Yachdav et al. 2014). Additionally, we used RegRNA (http://regrna.mbc.nctu.edu.tw/) for those synonymous SNPs, to determine the potential effects at mRNA level, stability, or gene expression regulation (Chang et al. 2013). ALDB, a domestic-animal long non-coding RNA database, was used to identify possible non-coding RNA, due to the presence of one of the SNP analyzed in this study, localized in a non-coding DNA region (Li et al. 2015). Also the MEME suite (Bailey et al. 2009) was used to identify possible motifs represented in our sequence as well as to predict potential effects of the SNPs changing the structure of a DNA motif. Multiple Em for Motif Elicitation (MEME) (Bailey and Elkan 1994), Gene Ontology for Motifs (GOMo), (Buske et al. 2010), and Motif Comparison Tool (Tomtom) (Gupta et al. 2007) tools were used to identify potential motifs in our sequence and analyze the gene ontology (GO) enrichment of this motifs by comparisons with previously described motifs.

Results

In order to focus the analysis on the productive traits and reduce the number of genes analyzed from thousands to few hundreds, only those probesets contained in the Affymetrix porcine expression microarray whose expression showed significant correlation with at least one of the seven phenotypes of interest were selected. A total of 820 probesets were selected, showing correlations between 0.32 and 0.66 (p value <0.001, q value <0.002) (Supplemental Table 2). Gene probesets were annotated using

NetAffx tool. One probeset per gene was chosen, the one showing the highest expression level. In total 776 gene-unique probesets were used for the eGWAS.

The eGWAS was carried out using GenABEL package among each of the 776 probesets with the filtered SNPs. The results revealed 954 associations between 880 SNPs with expression levels of 42 genes (eTAS) (Supplemental Table 3). These eTAS corresponded to 63 regions or eQTLs for 36 different transcripts, containing between 2 and 209 eTAS. From the total number of eQTLs 15 were *cis*-associations and 48 *trans*-associations. The eQTL regions were identified on every autosome except on SSC10, SSC12, SSC16, and SSC18 (Table 1), showing the higher association for R33-*trans* (SSC8) and R1-*cis* (SSC6). This analysis validated relevant associations such as the association between Insulin-Like Growth Factor 2 (*IGF2*) expression with R5-*trans* (28 SNPs) and R9-*trans* (2 SNPs) regions on SSC2. A total of 2630 genes were identified within these 63 eQTLS, containing candidate genes as *PTEN* (*Phosphatase and Tensin Homolog*, located on SSC14:108911519–109003081) associated with muscle development, *FADS1* (*Fatty Acid Desaturase 1*, located on SSC2:9,247,472–9,263,631), or *CTNNB1* (*Cadherin-Associated Protein, Beta 1*, located on SSC13:27,623,128–27,667,302).

We focused our further studies on those eQTLs (*cis*- and *trans*-associated) overlapping with QTLs previously detected in the same animal material (Óvilo et al. 2000; Varona et al. 2002; Mercadé et al. 2005; Fernández et al. 2012, 2014). Those were located on SSC4, SSC9, SSC13, and SSC17 (Table 2). These associations implicate four different expression probesets:

The *Ssc.7190.1.S1_at* probeset for QTL regions R19-*trans* and R35-*trans*, which corresponds with the *BUB1B* gene (ENSSSCG00000030580), SSC1:146,304,943–146,312,662. The *BUB1B* is associated with the proliferative capacity of muscle cells (Guntani et al. 2011).

The *Ssc.7666.1.A1_at* probeset for region R60-*cis*, which corresponds to *PSMF1* gene (ENSSSCG00000020887), SSC17:38,667,378–38,711,350. The PSMF1 interest lies in its interaction with *INS* (Insulin), *TGFB1* (Transforming Growth Factor Beta 1), and *CDKN1A* (Cyclin-Dependent Kinase Inhibitor 1A) which is related with myocyte terminal differentiation in muscle development (Guo et al. 1995; Qin et al. 2012).

The *Ssc.21242.1.S1_at* probeset for region R61-*trans*, which corresponds to *CTNNBL1* gene (ENSSSCG00000021553), is involved in basal metabolism and previously related with carcass traits in different species (Espigolan et al. 2015).

The *Ssc.10589.1.A1_at* probeset is located in a non-coding region. Current annotation indicates that this probeset corresponds to a long intergenic non-coding RNA (lncRNA), identified in the ALDB database (http://www.ibiomedical.net/aldb/) as *ALDBSSCG0000001928*, and located at SSC13:80,720,757–80,739,741. The LDB-SSCT0000003202 transcript spans 8,299 bp and two exons. This lncRNA is located in a region that overlaps with a high number of QTLs previously described on PigQTL database, as mainly associated with average daily gain (Hu et al. 2005, http://nhjy.hzau.edu.cn/kech/swxxx/jakj/dianzi/Bioinf8/Animal/Animal8.htm).

SNPs analysis

Candidate genes

A total of 44 positional and functional candidate genes for those QTLs overlapping with eQTLs were selected for candidate polymorphism search (Table 3). Polymorphism search was conducted taking the advantage of our previous SNP identification study based on an RNA-Seq assay performed on the same animal material (Martinez-Montes et al. 2016). We identified 49 SNPs in 13 of the 44 candidate genes. After validation and potential impact evaluation, a total of 20 SNPs located on 10 unique genes

Table 2 Identified eQTL overlapping with previous QTL

eQTL	Chr	Start (Mb)	End (Mb)	QTL	Trait	Start (Mb)	End (Mb)	Reference
R19	4	128,983,125	129,016,956	4	Fatness	129	134	Varona et al. (2002) and Fernández et al. (2012)
R35	9	118,021,130	120,667,155	9	Premium cut yield	120	127	Fernández et al. (2012)
R44	13	78,219,919	84,277,803	13	Premium cut yield	83	91	Fernández et al. (2012)
R45	13	87,702,788	89,919,771	13	Premium cut yield	83	91	Fernández et al. (2012)
R60	17	38,047,955	41,558,311	17	Fatness	39	44	Fernández et al. (2012)
R61	17	40,556,315	41,558,311	17	Fatness	39	44	Fernández et al. (2012)

Overlapping between eQTL identified in the current study and phenotypic QTL identified in previous studies in the IBMAP experimental population

Table 3 Positional candidate genes identified within the selected eQTLs

eQTL	Chr	Start (Mb)	End (Mb)	Candidate genes
R35	9	118,021,130	120,667,155	*LAMB1, NRCAM, LOC100623945, ENSSSCG00000025643, ZNF398, ZNF425, ZNF786, PDIA4, EZH2, CUL1*
R44	13	78,219,919	84,277,803	*LOC100738707, ACAD9, RAB7A, GATA2, MGLL, MCM7, PLXNA1, TXNRD3, CHST13, ACAD11, ICA, RYK, LOC100524398*
R45	13	87,702,788	89,919,771	*RBP2, RBP1*
R60	17	38,047,955	41,558,311	*FKBP1A, TCF15, TRIB3, LOC100519985, SOX12, ID1, BCL2L1, ENSSSCG00000022547, MYLK2, POFUT1, PLAGL2, ASXL1, COMMD7, DNMT3B, BPIFB2, BPIFB6, BPIFB4, BPIFB3*
R61	17	40,556,315	41,558,311	*POFUT1, PLAGL2, ASXL1, COMMD7, DNMT3B, BPIFB2, BPIFB6, BPIFB4, BPIFB3*

Genes located within each of the eQTLs selected by overlapping with QTLs

(Table 4) were selected for genotyping and association analyses in the backcrossed individuals (*ZNF786, ACAD11, RYK, MGLL, TRIB3, PDIA4, LAMB1, RBP1, TXNRD3*, and *ICA*):

The *MGLL* gene encodes a monoglyceride lipase that has been associated with fatty acid uptake and oxidation in pig intramuscular fatty acid composition in the *longissimus thoracic* muscle (Pena et al. 2013).

The *TXNRD3* encodes for thioredoxin reductase 3 that was shown to affect adipocyte differentiation through Wnt signaling pathway (Kipp et al. 2012).

The *ACAD11* gene that encodes an acyl-CoA dehydrogenase that was shown to be in association with variation in residual feed intake in beef cattle (Karisa et al. 2013).

The *RYK* genes encode a receptor-like tyrosine kinase that mediate muscle attachment in drosophila melanogaster via Wnt interaction (Lahaye et al. 2012).

The *RBP1* gene encodes a retinol-binding protein that regulates adipogenesis in mice (Zizola et al. 2010).

The *TRIB3* genes encode tribbles pseudokinase 3 that was shown to be in association with meat quality and production traits in Italian heavy pigs (Fontanesi et al. 2010).

Table 4 Description of candidate SNPs selected and analyzed within candidate genes

SNP ID[a]	MAF	Chr	Genomic position	Location	Predicted SNP effect
ss2031475799	0.25	9	118452493	exon 26	–
ss2031475800	0.34	9	118456942	intron (ex24–ex25)	–
ss2031475801	0.47	9	118475910	exon 17	–
ss2031475802	0.44	9	120182492	exon 3	AA change
ss2031475803	0.43	9	120183584	exon 4	miRNA Target gain
ss2031475804	0.38	9	120198443	exon 1 (5′ utr)	–
ss2031475805	0.44	9	120215042	exon 8	–
ss2031475806	0.15	13	80086134	exon 8 (3′ utr)	–
ss2031475807	0.34	13	80086582	exon 8 (3′ utr)	–
ss2031475808	0.34	13	80691943	intron (ex8–ex9)	–
ss2031475809	0.34	13	80691962	intron (ex8–ex9)	–
ss2031475810	0.22	13	80692144	intron (ex8–ex9)	ESE change
ss2031475811	0.22	13	80693764	exon 7	–
ss2031475812	0.22	13	81350505	exon 13	AA change
ss2031475813	0.49	13	82370845	exon 2	AA change
ss2031475814	0.03	13	82824584	exon 14 (3′ utr)	ESE loss
ss2031475815	0.21	13	82846842	exon 12	–
ss2031475816	0.32	13	88073665	exon 1 (5′ utr)	–
ss2031475817	0.25	17	39530285	exon 4 (3′ utr)	–
ss2031475818	0.06	17	39584558	intron (ex2–ex3)	–

Genomic localisations, minimum allele frequencies, and predicted effects are shown for candidate SNP

ESE exonic splicing enhancer

[a]Position within gene

The *LAMB1* gene encodes beta laminin 1, which is associated with skeletal muscle development in human (Wewer et al. 1997).

The *PDIA4* gene encodes a protein disulfide isomerase that is associated with HSP90 activity in muscle differentiation (Garcia de la Serrana and Johnston 2013).

The 20 SNPs were successfully genotyped in the backcrossed animals showing MAFs ranging from 0.03 for *ss2031475815* to 0.49 for *ss2031475813*, most of the SNPs showed intermediate frequencies (Table 5).

Association analysis

Most of the selected SNPs (65%) showed intermediate frequencies in the backcross population, MAF >0.25, optimal values for association analysis (Tabangin et al. 2009), and only two SNPs showed very low frequencies [*ss2031475814* (MAF = 0.03) and *ss2031475818* (MAF = 0.06)] and were discarded for the association analyses. Also linkage disequilibrium estimates were calculated for closely linked SNPs. Complete linkage was found for *ss2031475811, ss2031475810*, and *ss2031475812*, and between *ss2031475802* and *ss2031475803* polymorphisms.

Specific association analyses of each candidate SNP with the corresponding probeset expression level were conducted, in agreement with eGWAS results (Table 5). In addition, association analyses were done for the candidate SNPs with the phenotypic traits (SW, HW, BLW, BW150, IMF, BFT75, and BFS).

Significant association with gene expression measures were found for *ss2031475813, ss2031475806, ss2031475807, ss2031475816, ss2031475814, ss2031475817, ss2031475811, ss2031475809*, and *ss2031475808*. The whole results could be grouped into two different clusters conditional on the affected gene expression: Ssc.10589.1.A1_at probeset, representing *ALDBSSCG0000001928* lncRNA expression and Ssc.7666.1.A1_at probeset, representing *PSMF1* gene expression.

Within the first cluster, the *ss2031475813, ss2031475806, ss2031475807, ss2031475816, ss2031475814, ss2031475811, ss2031475809, ss2031475808* SNPs showed association with the Ssc.10589.1.A1_at expression levels. All eight SNPs are located in R44-*cis*, showing a decrease of expression levels between 0.437 and 0.918 (Table 5). Only *ss2031475816* SNP, which is located in R45-*trans* (trans-association), reported an increase of *Ssc.10589.1.A1*_at expression levels in 0.779 with a standard error (SE) of ±0.083. For the second cluster, only one SNP was associated with the expression levels of *PSMF1* gene, *ss2031475817*, which increases expression in 0.224 (±0.062).

Regarding the association analysis results for the production traits, the *ss2031475809*, which showed the higher association with expression levels of SSsc.10589.1.A1_at probeset, showed also the most significant effect on BW150, increasing animal weight in 2.66 kg (±1.07) (Table 5). Additionally, it also revealed effects on HW and BLW increasing weight in 156 g (±88) and 148 g (±79), respectively. Besides *ss2031475808, ss2031475814*, and *ss2031475806* SNPs showed associations, p value <0.05, with BW150 trait. Suggestive effects ($p < 0.10$) of *ss2031475816* on BW150, and *ss2031475817* on HW could also be reported. Here, it should be noted that the animal number is a power limitation in the identification of significant effects in the association analysis (Hong and Park 2012).

The linkage disequilibrium estimates for *TXNRD3, MGLL, ICA*, and *RBP1* SNPs (Fig. 1) revealed a significant linkage block composed by two SNPs of *MGLL* and the three SNPs of *TXNRD3* (*ss2031475806, ss2031475807, ss2031475808, ss2031475809*, and *ss2031475810*), two

Table 5 Significant association results of the analyzed candidate SNPs on gene expression and phenotypic traits

SNP_ID[a]	Gene probeset	W150D	HW	SW	BLW
ss2031475813	−0.437 (0.127)[¥]	1.860 (1.322)	−0.041 (0.107)	−0.022 (0.057)	0.000 (0.096)
ss2031475806	−0.599 (0.153)[¥]	1.365 (1.738)	0.015 (0.139)	0.000 (0.071)	0.017 (0.123)
ss2031475807	−0.781 (0.109)[¥]	2.710 (1.316)**	0.118 (0.092)	0.053 (0.056)	0.084 (0.081)
ss2031475816	0.779 (0.083)[¥]	−1.893 (1.148)*	−0.140 (0.089)	−0.054 (0.048)	−0.046 (0.081)
ss2031475814	−0.604 (0.120)[¥]	2.667 (1.316)**	−0.056 (0.109)	−0.048 (0.057)	−0.023 (0.096)
ss2031475817	0.224 (0.062)[¥]	1.878 (1.324)	0.161 (0.095)*	0.020 (0.062)	0.041 (0.086)
ss2031475810	−0.758 (0.110)[¥]	2.279 (1.365)	−0.007 (0.113)	−0.012 (0.058)	0.020 (0.099)
ss2031475809	−0.918 (0.071)[¥]	2.665 (1.072)[¥]	0.156 (0.077)**	0.0290 (0.052)	0.148 (0.070)**
ss2031475808	−0.708 (0.108)[¥]	2.651 (1.220)**	0.096 (0.088)	0.051 (0.055)	0.068 (0.079)

Results of the association analysis carried out for the 20 candidate SNPs with weight at 150 days, ham, shoulder, and bone-in-loin weights as phenotypic traits

[¥]Bonferroni correction ($q < 0.05$), **$p < 0.05$, *$p < 0.1$

[a]Position within gene

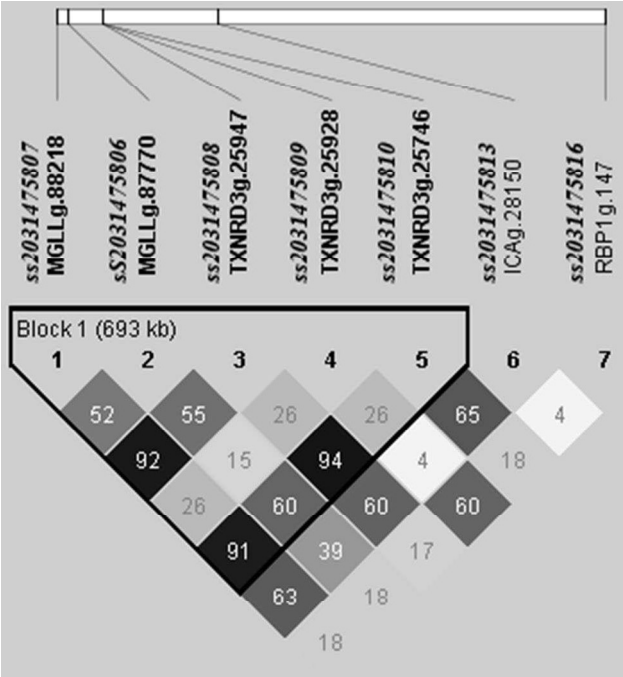

Fig. 1 Linkage disequilibrium (r^2) representation among the SNPs associated with Ssc.10589.1.A1_at expression levels

genes located very close in SSC13, at 594 kb distance. Four haplotypes were identified for these five SNPs: AAACC, AAATC, GGTTT, and GATTT. The same association analysis as those used with single SNPs were carried out for the haplotypes, trying to determine if the addition of genomic information to the analysis could better explain the effects than the individual SNPs. Nevertheless, the results were less significant. The association analysis of *ss2031475817*, the unique SNP associated with *PSMF1* gene expression, with the productive traits revealed effects on HW trait (Table 5).

In addition, few other SNPs located within these eQTL regions showing effects on production traits: *ss2031475801* showed effects on HW and BW150, *ss2031475800* on HW and SW, *ss2031475799* on HW, SW, and IMF, and *ss2031475814* on BW150 (results not shown). Some of these results may be relevant; however, due to the lack of association with probeset expression levels (the initial hypothesis of the current study), these results were not further studied.

Discussion

In the current study we focused our analyses on the identification of mutations that could affect expression levels of genes involved in porcine fat deposition and growth processes. In order to achieve this objective a genetical genomics study was conducted using the expression levels for 776 genes selected due to the correlation between expression levels and phenotypic traits, currently known as intermediate phenotypes, correlated with fat deposition and growth-related traits, in an eGWAS. This approach allowed us to identify a total of 954 significant associations between 42 genes and 880 eTAS. Moreover, we were able to validate interesting associations between SNPs and gene expression levels, such as those identified for *Insulin-Like Growth Factor 2* (*IGF2*), R5-*trans* that contains 28 SNPs associated with expression, at SSC2:16,416–10,979,357, and R9-*trans* containing 2 SNPs SSC2:162,084,552–162,298,086, where are reported to map the causal mutation affecting *IGF2* gene expression, involved in muscle development (Van Laere et al. 2003), fatty acid composition (Hong et al. 2015), and litter size (Muñoz et al. 2010).

As expected, the identification of SNPs affecting phenotypic traits is less precise than identifying association with expression levels directly, likely due to the most direct relation between gene expression and genomic information. Gene expression seems to be regulated in a simple way if we compared it with complex phenotypic traits. Nevertheless, the interpretation and biological relevance of the associations identified here need further analyses to unravel these complex regulation mechanisms.

Beyond the identification of associations between SNPs and gene expressions, the eGWAS has allowed us to identify 63 eQTLs. Although region size and gene content seem to be variable, a lot of information could be obtained from these genomic regions. eQTLs were identified in all autosomes except SSC10, SSC12, SSC16, and SSC18. The SSC13 showed 13 different eQTL regions associated with four different gene expression levels, ten of those were associated with *TXNRD3* expression. The regions identified on SSC13 covered almost 60% of total chromosome length, which could be explained by high linkage disequilibrium levels (Fig. 2) as previously reported (Saura et al. 2015). Positional and functional candidate genes were identified in some of these regions, allowing us to select potential genes underlying the identified eQTLs. Some of the genes are transcription factors previously associated with traits of interest such as the *FOXO1*, associated with adipogenesis in porcine preadipocytes (Yan et al. 2013), the *GATA2*, involved in adipogenesis (Szczerbal and Chmurzynska 2008), and the *RBL1* (p107), which has been proposed to regulate adipocyte differentiation (Scimè et al. 2005).

One of the challenges of this kind of studies is how to manage the great amount of results obtained from the eGWAS. Although a lot of interesting regions and genes were detected, the study was focused on those regions that overlap with phenotypic QTLs previously described in the same animal material. The eQTL regions at SSC4, SSC9, SSC13, and SSC17 overlapped with QTLs for

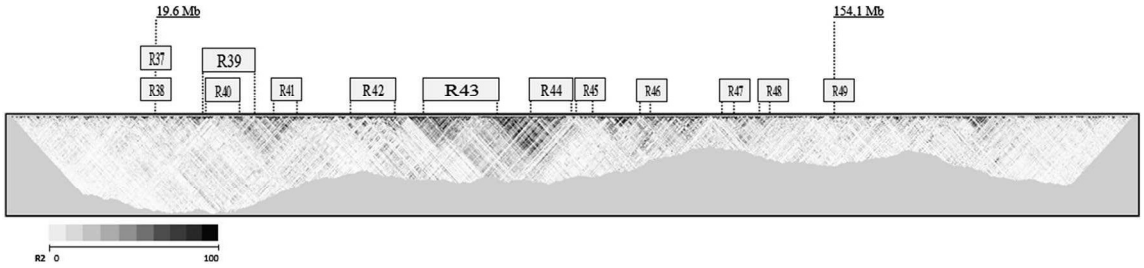

Fig. 2 Linkage disequilibrium heat map of eQTL regions on SSC13

fatness and premium cut yield (Varona et al. 2002; Fernandez et al. 2012). With this approach, some of the results remained unanalyzed but it brings interesting data for further studies.

In order to identify candidate mutations that could be underlying the selected eQTLs, SNPs identified in a previous RNA-Seq study were used (Martínez-Montes et al. 2016). This approach allowed us to select not only candidate SNPs located on those regions, but also SNPs that showed differential genotype between divergent groups for growth and fat deposition (Martínez-Montes et al. 2016). Merging both studies, SNP identification and GWAS results, we were able to focus our analysis on the identification of candidate causal mutations. The strength of this approach relies on the possibility of using different type of results in order to answer a common objective. Following this strategy, we were able to identify potential candidate genes that could be regulating the expression levels of three genes: *BUB1B*, ALDBSSCG0000001928, and *PSMF1*. Even more, candidate mutations associated with gene expression and production traits were also identified. One of the most interesting results is the association detected between *PSMF1* and *TRIB3* SNPs. Previous studies have reported the association of *TRIB3* polymorphisms with meat quality and production traits in Italian heavy pigs (Fontanesi et al. 2010) by reducing the fat levels and increasing weight. Moreover, the *PSMF1* interacts indirectly with *TRIB3* gene, via *AKT2* and *UBC* genes which have been previously associated with adipogenesis and muscle development (Pang et al. 2013; Ayuso et al. 2015). Among the most promising and novelty result is the identification of ALDBSSCG0000001928 lncRNA, whose expression seems to be associated with *TXNRD3* polymorphisms.

The analyzed *ss2031475809* could be the causal mutation affecting the expression levels of this lncRNA, and it appears to also be associated with body weight and premium cut yields. It should be also noted that several regions of SSC13 chromosome, ten different regions, are *trans*-associated with the same lncRNA expression levels (R37, R39, R41, R42, R43, R44, R45, R46, R47, and R49), but the most significant association corresponded to the *cis*-association of R44, which includes *ss2031475809*.

The *Ssc.10589.1.A1_at* probeset was firstly annotated within *TXNRD3* gene. Nevertheless, after annotation updates and deeper sequence analysis by basic local alignment searches with BLAST, the annotation confirmed that it represents a long intergenic non-coding RNA (lncRNA) gene annotated in the domestic-animal long non-coding RNA database (ALDB) as *ALDBSSCG0000001928* gene (*ALDBSSCT0000003202* transcript). This lncRNA is located close to *TXNRD3* gene, at 3 Kb of *ss2031475809*. Annotation data showed that *ALDBSSCG000000192* gene is located within a QTL region for several productive traits such as average daily gain, body weight, and back fat weight (pigQTL database).

Long non-coding RNAs (lncRNA) have been identified as chromatin regulators in different species and act following different strategies. For instance, the *XIST* gene, which is an lncRNA upregulated in one of the female X chromosomes of mice in early development, leads to transcriptional repression and important changes in chromatin composition. Nevertheless, it acts also for dosage compensation *roX* gene in Drosophila melanogaster, increasing transcription on the single male X chromosome (Rutenberg-Schoenberg et al. 2016). But several other roles have been attributed to lncRNAs such as transcriptional regulation and post-transcriptional control (Angrand et al. 2015). In the current study, we hypothesize that *ALDBSSCG0000001928* lncRNA could be regulating expression levels, through transcriptional repression, of surrounding genes such as *MGLL* and *TXNRD3* (negative significant correlation, −0.43 and −0.42 was detected with lncRNA expression, respectively).

The potential mechanism explaining the relation between *ss2031475809* SNP and *ALDBSSCG0000001928* lncRNA was explored using Motif Comparison tool from the MEME suite. Potential motifs including or close to *ss2031475809* were identified (Fig. 3). The most relevant corresponded to the motif CAC[A/C]T[A/G]AG, which involves conservation in the *ss2031475809* position indicating its high functional relevance. Additionally,

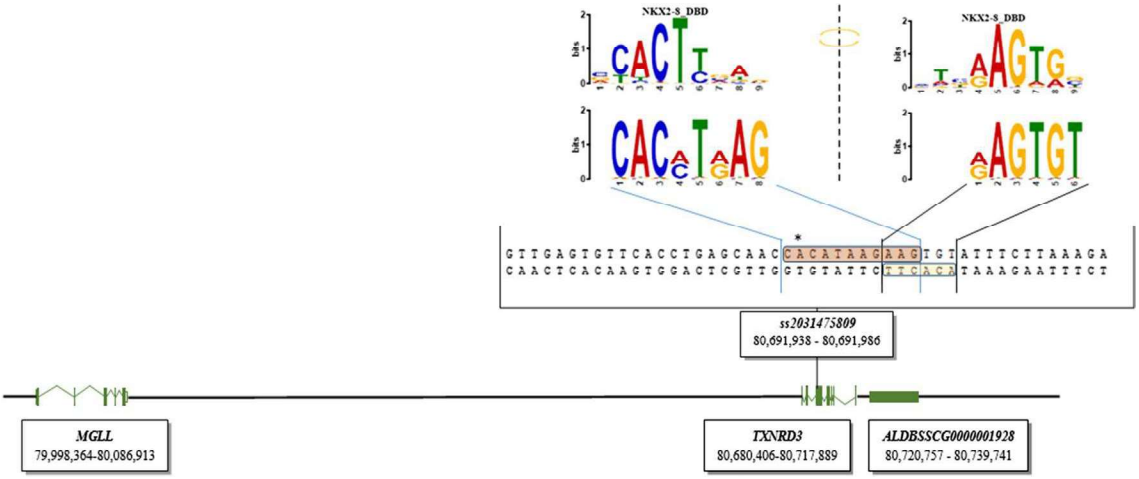

Fig. 3 Genomic organization of *MGLL, TXNRD3* (*ss2031475809*) and ALDBSSCG0000001928 lncRNA genes. Representation of NKX2-8 DBD motifs within *TXNRD3* gene sequence

two more motifs (similar to NKX2-8 DBD) were predicted (Fig. 3). Using the GOMo tool to scan promoters to determine if the identified motif is significantly associated with genes linked to one or more Genome Ontology (GO) terms, we were able to observe enrichment, among human gene catalogue, for olfactory receptor activity (GO:0004984), sensory perception of smell (GO:0007608), and sensory perception of chemical stimulus (GO:0007606). These terms involve genes such as taste receptors likely mediating growth and fatness processes (Ren et al. 2009) and olfactory receptors, which have been studied in porcine due to the possible relevance in pig over other species (Nguyen et al. 2012) and their relation with gastrointestinal tract in pigs (Priori et al. 2015). These results support *ss2031475809* as candidate mutation, to regulate *ALDBSSCG0000001928* lncRNA expression, which can be involved in the transcriptional regulation of *MGLL* and *TXNRD3*, affecting productive and meat quality traits (Pena et al. 2013; Puig-Oliveras et al. 2016).

In conclusion, we were able to identify 63 eQTL regions affecting 36 transcript expressions, which overlapped with phenotypic QTLs on chromosomes SSC4, SSC9, SSC13, and SSC17. Also candidate genes on these regions, and candidate SNPs obtained from RNA-Seq data were analyzed. One of the most relevant results is the identification of *ALDBSSCG0000001928*, a long non-coding RNA, whose expression seems to be correlated with premium cut yield. In silico domain annotation and association analysis support the role of TXNRD3 polymorphisms as potential candidates to regulate *ALDBSSCG0000001928* expression. This lncRNA could be involved in the transcriptional regulation of genes surrounding it, as other lncRNA are reported to, affecting productive and meat quality traits.

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Artículo III

Using genome wide association studies to identify common and specific QTL in three different genetic backgrounds based on Iberian pig breed

Martínez-Montes AM, Fernández A, Muñoz M, Noguera JL, Folch JM, Fernández AI Using genome wide association studies to identify common and specific QTL in three different genetic backgrounds based on Iberian pig breed. PlosOne [en preparación]

Using genome wide association studies to identify common and specific QTL in three different genetic backgrounds based on Iberian pig breed

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Short title: Common and specific porcine QTL in three different genetic backgrounds

ABSTRACT

One of the major limitation for the application of QTL results in pig breeding and QTN identification has been the limited number of QTL effects validated in different animal material. The aim of the current work was to validate QTL regions through independent and joined genome wide association analyses for growth, fatness and premier cut yields in three different genetic backgrounds based on Iberian pigs, which has a major role in the analysis due to its high productive relevance. The results revealed 22 common QTL regions located on nine different autosomes, 15 identified in at least two of the independent backcrosses and the merged dataset, and seven regions identified in the merge dataset (joined analysis) and no identified in the independent analyses. Moreover, 58 QTL regions were specifically identified in one of the three backcrosses. Beyond identifying and validating QTL, candidate genes and mutation within the most interesting regions have been explored using functional annotation, gene expression data and polymorphisms identification from RNA-Seq data. A total of 105 candidate genes were selected, 24 genes for the common QTL regions and 81 genes for specific backcross QTL regions, and a list of polymorphisms on genes *CAST, IL4R, CAV1, MLH1, HP, SELL, SELP* and *CXCR4* are proposed as candidate mutation to be further investigated.

INTRODUCTION

QTL identification is one of the most relevant approaches used in livestock genomic studies in order to understand the genetic architecture that regulates complex productive traits. To date, different porcine breed schemes had been used for QTL scanning, from simple designs including purebred populations such as Pietrain, Landrace or Duroc [1], [2] to more complex schemes, mating different breeds in order to compare animals with diverse phenotypes, as Duroc x Pietrain [3], [4], Iberian x Landrace [5] or three-way crosses such as Duroc x (Landrace x Large White) [6]. These studies have reported a large number of QTL for different productive traits such as growth (1,328), fat composition (1,311), drip loss (1,071), average daily gain (568), average backfat thickness (332) or intramuscular fat content (244) (PigQTLdb) [7].

In spite of the great amount of QTL identified in multiple pig breeds, the

application of the results in pig breeding and the identification of causal genes and mutations (QTN) has not been very successful. One of the major limitations had been the low number of available markers [8], [9]. However, this issue has been settled with the development of high-density genotyping platforms, which provide a high number of markers along the genome, allowing us to QTL fine-map and to conduct genome-wide analysis (GWAS) [10], [11]. Another major limitation for QTN identification has been the limited number of animals employed in the analyses [12]–[14], reporting unreliable results that cannot be validated in different genetic backgrounds. So far, few porcine QTL regions related to productive traits have been confirmed in different animal material, except the QTL around *LEPR* region for growth, fatness and meat quality traits identified in Iberian x Landrace cross [15], and validated in Iberian x Meishan cross [16] and Duroc populations [17], [18], the FAT1 QTL located on SSC4 associated with fatty acid metabolism validated in Meishan x Large White, Iberian x Landrace and Wild Boar intercrosses [19]–[23] and the QTL located on SSC12 for fatty acid composition was validated in different Iberian and Landrace cross populations and in purebred Duroc [17], [24]–[27].

The aim of the current work was to validate QTL regions through GWAS analyses for growth, fatness and premier cut yield in three different genetic backgrounds F1 (Iberian x Landrace) x Landrace (BC_LD), F1 (Iberian x Duroc) x Duroc (BC_DU) and F1 (Iberian x Pietrain) x Pietrain (BC_PI) backcrosses. Here, Iberian background had a major role in the analysis due to the high productive relevance of this breed [28]. Beyond identifying and validating QTL, candidate genes and polymorphism within the most interesting regions have been proposed and explored.

MATERIAL AND METHODS

Animals: Phenotypic and genotypic data used in this study belong to three different experimental backcrosses; F1 (Iberian x Landrace) x Landrace, F1 (Iberian x Duroc) x Duroc and F1 (Iberian x Pietrain) x Pietrain. Schematic representation of the backcross generation is shown in Figure 1.

All animal procedures were performed according to the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 86/609 about the protection of animals used in experimentation.

Phenotypic data: Six traits related with growth, fatness and premium cut yields recorded for all three backcross pigs were analyzed (Table 1). These traits were: body weight at 150 days of mean age (BW150), backfat thickness measured at 75 kg of live weight (BFT75), weights of premium cuts, such as hams (HW) shoulders (SW) and loin bone-in (LBW) and intramuscular fat content (IMF) measured in *Longissimus dorsi* samples at slaughter as described in Fernandez et al. (2012) [26].

Genotypic data: Two different genotyping platforms were used, BC_LD and BC_PI backcrosses were genotyped with the platform PorcineSNP60 BeadChip (Illumina, Inc.) [29], containing around 64,232 SNPs. GenomeStudio software (Illumina, Inc.) was employed to visualize, edit, standard quality filter and extract genotyping data. Backcross BC_DU was genotyped with Axiom® Porcine Genotyping Array (Affymetrix, Inc.) [30], containing around 658,692 SNPs. Axiom™Analysis Suite 2.0 was employed to visualize, quality filter and extract genotype data. For individual analysis of each backcross, additional data filtering was carried out using GenABEL package [31] in R environment, those polymorphisms with a minimum allele frequency (MAF) < 0.05 were discarded for further analysis. In order to be able to compare data from the two platforms SNPchimp v.3 [32] was used to select those SNPs overlapping between both.

GWAS analysis: Genome-wide association studies were conducted for each backcross independently and in a joint analysis of the three backcrosses. The analyses were performed with GenABEL R package using the following the model:

$$y_{ijk} = S_i + B_j + bx_k + \sum_l \lambda_{lk} a_l + u_k + e_{ijk}$$

where y_{ijk} is the trait value of kth individual, S_i and B_j are fixed effects for sex and batch respectively and b is the slaughter weight regression coefficient only included for HW, SW, LBW and IMF traits. Additive effect of the SNP is a_l and λ_{lk} is the indicator related with the number of copies of the lth allele (0, 1 or 2), u_k is the infinitesimal effect of the kth individual, e_{ijk} is the random residual term. For the joint analysis (merged database) the backcross was included as fixed effect. Significance thresholds were estimated using QQ-Plot [33].

Region analysis: QTL regions were determined by two or more SNPs significantly associated with each phenotypic trait, close each other to a

maximum distance of 3Mb. Additionally, regions identified within a maximum distance of 15Mb were considered as the same region. Gene content of these QTL was determined using Biomart tool from the Porcine Ensembl database. In order to validate association results and to better determine the QTL effects, the most significant marker of each QTL region was analyzed using the previous quoted model with Qxpak 5.0 software [34].

Candidate Genes: In order to identify potential candidate genes associated with growth, fat deposition and premier cut yield, the function of genes within each of the selected regions were examined using VarElect [35] and STRING [36] databases. Moreover, additional information of gene expression data from a previous study in BC_LD [37] and a parallel RNA-Seq study conducted in the BC_LD and BC_PI pigs with divergent phenotypes for the same analyzed traits [38] was also used. Genes mapped within the selected QTL regions showing significant differential expression (q-value< 0.05 and fold change> 1.5) were also retained.

SNP calling: RNA-Seq data obtained from liver, hypothalamus and *Longissimus dorsi* muscle samples, from pigs with divergent phenotypes for growth and fatness on each backcross [38], [39] was used for SNP calling, in order to identify candidate polymorphism within the selected candidate genes. RNA-Seq data filtering, mapping and SNP calling was carried out with CLC Genomics Workbench (www.clcbio.com). Reads were mapped against pig reference genome (Sscrofa10.2). Mapping parameters were set at a cost of two for mismatches per read, cost of three for insertion or deletions, a length fraction of 0.9 and a similarity fraction of 0.8. Quality-based variant detection tool from CLC Genomic Workbench, based on neighborhood quality [40], [41], was used to perform SNP calling, setting a neighborhood radius of 5, minimum neighborhood quality of 15 and minimum central quality of 20, a minimum coverage of 3 and a 20% of minimum variant frequency.

The SNPs were classified using VENNY 2.1 tool [42] as: 1) those SNPs identified in genes located in common regions observed in all the backcrosses, and 2) those SNPs segregating in the specific backcross where backcross-specific QTL regions were detected (S4 Table). Besides, the functional relevance of these SNPs was analyzed with VeP tool (Variant effect Prediction, Ensembl) in

order to identify potential candidate mutations (S5 Table).

RESULTS

SNP data:

For the GWAS analyses only SNPs overlapping between genotyping platforms (60K and 650K) for each backcross were retained. A total of 39,279 SNPs in 102 BC_LD pigs, 38,684 SNPs in 139 BC_DU pigs, and 38,891 SNPs in 144 individuals BC_PI pigs were selected. The merged dataset contained 40,929 SNPs in 385 backcrossed pigs. The SNPs showing a MAF ≥ 0.05 were around 80% in each backcross and 97% in the merged dataset. Approximately a 50% of them showed intermediate frequency (MAF ≥ 0.25) in each backcross, and 61% in the merged dataset (S1 Figure). These data revealed a similar allele frequencies distribution across all the animal material, therefore none restraining effects related to SNP informativity would be expected in the QTL region identification, although it could be found for specific genomic regions.

GWAS analysis:

The GWAS analyses for BW150, BFT75, HW, SW, LBW and IMF traits were carried out for each backcross independently and for the merged dataset. Significance thresholds were estimated using QQ-Plot approach (S2 Figure) and results were represented using Manhattan Plots (S3 Figure). The results from the merged analysis reported a total of 1,790 significant associations of 1,706 trait associated SNPs (TAS) with the six traits, 1,622 associations of 1,534 TAS in the independent BC_LD analysis, 858 associations of 990 TAS in the independent BC_DU analysis and 613 associations of 569 TAS in the independent BC_PI (Table 2).

Region analysis:

Region identification revealed 147 QTL regions in the merged dataset, 138 regions in the BC_LD, 110 regions in the BC_DU and 72 regions in the BC_PI. Overlapping among the four GWAS analyses lead to the identification of 15 common regions identified in at least two of the independent backcrosses and the merged dataset, seven regions identified in the merged dataset and no identified in the independent analyses (Table 3), both corresponding to common QTL regions, and 58 regions specifically identified in one of the three independent GWAS (Table 4, 5, 6).

The common QTL regions, located on SSC1, SSC2, SSC3, SSC6, SSC9, SSC13, SSC15 and SSC16, were associated with all phenotypic traits except for BFT75 (Table 3). The seven QTL regions identified in the merged dataset but not in the individual analyses were localized on SSC1, SSC13, SSC15 and SSC18, associated with BW150, HW, SW and IMF (Table 3). The 29 regions identified specifically in the BC_LD were located on SSC1, SSC2, SSC3, SSC4, SSC5, SSC6, SSC7, SSC8, SSC11, SSC14, SSC16, SSC17 and SSC18, associated with all six phenotypic traits (Table 4). The 12 regions identified specifically in the BC_DU were located on SSC1, SS2, SSC3, SSC4, SSC6, SSC7, SSC8, SSC11, SSC17 and SSC18, and showed association with BW150, BFT75, HW and SW, and (Table 5). Finally, the 17 regions identified specifically in the BC_PI were located on SSC2, SSC4, SSC6, SSC7, SSC8, SSC10, SS11, SSC12, SSC14, SSC15, SSC16 and SSC18, and showed association with BW150, LBW and IMF (Table 6). Additive effect estimations carried out with the most significant SNP of each region using Qxpack are shown in Table 3 for the common regions and in Tables 4, 5 and 6 for the backcross-specific regions.

Candidate Genes and SNPs analyses:

In order to identify potential candidates, genes located within significant regions were annotated using BioMart tool. As a result, 931 genes were identified on the common regions, 834 genes on the regions identified in at least two of the independent backcrosses, 147 genes on the regions of the merged dataset analysis, and 2,864 genes on the backcross-specific regions. To reduce the candidate gene list towards a more affordable dataset, a criteria based on functionality and gene expression differences from parallel RNA-Seq studies was followed to prioritize them [37], [38]. A total of 105 candidate genes were selected, 24 genes for the common regions, and 81 genes for specific backcross regions (Table 7, 8, 9, 10).

SNP calling:

Candidate SNPs were identified from RNA-Seq data analyses [38], [39]. A total of 1,452,878 were identified, however, only those SNPs within candidate genes and showing segregation in all the backcrosses were retained (at least 25% of the individuals carried the alternative allele). SNPs were identified for the following candidate genes; *PRKDC*, *SELL*, *SELP*, *KARS*, *HP*, *CXCR4*, *MUSK*, *ASS1*, *SUCLA2*, *KRT8*, *DSP*, *EGFLAM*, *CAST*, *ERAP1*,

PAM, *IL4R*, *MLH1* and *CAV1* (S4 Table). The VeP ensemble tool allowed the annotation of 11 variants at 5'/3' UTRs, 15 upstream and downstream variants, 37 intron variants 28 synonymous variants and 11 missense variants. From the 104 variants, the most promising ones were those located on genes *CAST, IL4R, CAV1, MLH1, HP, SELL, SELP* and *CXCR4* (Table 11). The *Calpastatin* (*CAST*) gene, encodes an endogenous calpain which expression has been associated with carcass and meat quality traits in several previous studies [43]–[45]. The *Interleukin-4 receptor subunit alpha precursor* (*IL4R*) gene, encodes an alpha chain of the interleukin-4 receptor, which is a member of the *JAK*/*STAT*. The *IL4R* expression is up-regulated in response to strength training in human [46] and it has been reported to contain a region for growth signal transduction [47], [48]. The *Caveolin 1* (*CAV1*) gene encodes a scaffold protein from the Caveolin family that plays important roles in different cellular process such as regulation of cell morphology and gene expression in muscle cells. The *CAV1* has been proposed as a potential candidate for carcass traits and could help clarifying caveolae signaling role in fat deposition [49]. The *MutL homolog 1* (*MLH1*) gene encodes a DNA repair protein, previously described to content a SNP highly significant associated with milk yield, but also with fat and protein in Holstein cattle [50]. The *Haptoblogin* (*HP*) gene encodes a protein associated with type 2 diabetes, [51]. The *Selectin L/P* (*SELL/SELP*) genes encode selectin family proteins, which are cell adhesion molecules associated with fatty acid composition [52]. Moreover, the *SELP* gene has been associated with long fatty acid absorption [53] affecting endothelial function [54]–[56]. Finally, The *C-X-C Motif Chemokine Receptor 4* (*CXCR4*) gene encodes a CXC chemokine receptor likely associated with muscle development through *CXCR4*/*SDF1* interaction [57]. A narrow relation between this gene and muscle could be inferred owing to miR-133b [58].

DISCUSSION

QTL detection and validation

The main objective of the current study was to validate QTL among different genetic backgrounds. In order to achieve this objective, a GWAS study was designed specifically for this purpose, in which four different pig breeds were employed in three experimental backcrosses, highlighting the productive

value of the Iberian breed versus the widely employed commercial pig breeds as Landrace, Duroc and Pietrain. The relevance of Iberian pig in meat production relies on the ability to produce high quality dry-cured products [59], due to particular characteristics associated with fattening, meat and growth process [28]. Iberian pigs have high fat deposition and desaturation levels, with particular fatty acid profiles and tend to accumulate infiltrated fat in muscle mass [60]. Leptin resistance is a characteristic property identified in this breed, and is associated with high food intake [15], [16].

The approach used in the current study, enabled us to identify a great amount of specific associations between SNPs and productive traits in each of the backcrosses, 1,622 for BC_LD, 858 for BC_DU and 613 for BC_PI, setting a great base for further analyses. Besides identifying TAS, we were able to define a total of 458 QTL regions in all the material, some of them being specific for each of the backcrosses, 29 for BC_LD, 12 for BC_DU and 17 for BC_PI. But more importantly, the analyses allowed us to validate 22 common QTL regions, located on nine different autosomes and associated with BW150, HW, SW, LBW and IMF traits. Most of these QTL regions are expected to be related with the Iberian breed characteristics, as they are present in at least two of the three backcrosses and in the merge dataset, all of them sharing Iberian genomic background. In fact, the results revealed that most of the associations are related to body-weight and yields related traits, mainly with HW, which agree with previous studies that identified smaller and lighter new-born pigs and lower ham yields in growing pigs in purebred than Duroc x Iberian crossed animals [28], [61]. The identification of 12 out of the 22 common QTL associated with HW (Table 3), may indicate the potential of these genomic regions to content relevant information of genetic basis that regulates body development.

The comparison with QTL previously described (QTLdatabase) revealed that 19 of the common QTL regions identified in the current study overlap with previously described QTL. This validation in another animal material increases the reliability of our results. For example, the MR1 region overlaps with a QTL for body weight at birth [62]; MR2 overlaps with a QTL identified for ham-weight [63]; CR8 overlaps with QTL for ham-weight [64]; and MR3 overlaps with a QTL for intramuscular fat content [65]. The remaining three

common QTL regions, CR9, CR11 and CR13 does not overlap with any previously described QTL according with PigQTL database. These QTL regions could provide novel relevant information relative to HW and IMF regulation in porcine. In the same way, some of the results obtained in the current study reveal higher precision on the QTL localization. For instance, the regions CR6, CR7, and CR12 overlap with QTL previously described [66]–[70] but with slightly lower span ranges.

Additionally, the effect of the sample size in QTL scans can be shown in the current analyses. A total of 147 QTL were detected employing the merge dataset, seven QTL out of them were identified specifically in the merge dataset but no in the independent analyses, supporting that increasing the individual number, even from different genetic backgrounds, improves the statistical power, allowing the identification of QTL regions that could not be identified in the independent analyses.

As previously mentioned, regions only identified in one of the three backcrosses were also selected due to the potential relevant information for specific pig breeds. For BC_LD, 29 QTL associated with the six phenotypic traits were detected, 24 of them related with cut-yields (HW, LBW and SW) and BW150 and with predicted effects showing an increase in BW150 and LBW (Table 4). These results agree with Landrace characteristics, since this breed has higher average daily gain and body weight compared with Iberian breed and also with the fact that Landrace breed have long bodies and one of the longest carcasses [71], [72], as it can be shown in Table 2 registers. For BC_DU, 12 QTL associated with four of the six traits, BFT75, HW, SW and BW150 were detected, remarking those regions associated with BW150, HW and SW which effects can be summarized in an increase of BW150 and decrease of SW and HW (Table 5). As previously mentioned, the Iberian x Duroc crossed animals showed significant differences for body weight and ham yields comparing with purebred Iberian [28], [61]. For BC_PI, 17 QTL associated with only three traits were detected, eight of them associated with IMF and eight with LBW, being those associated with IMF the most significant ones due to the genomic content and number of associated SNPs, and also supported by the predicted effects of these regions decreasing IMF fat content (Table 6). These results agree with the extreme differences between Iberian and Pietrain

breeds for IMF, as Pietrain pigs have a faster growth and lower levels of IMF [73]. To verify the actual backcross specificity of these QTL regions, the allele frequencies of the SNPs contained in the regions were examined. For example, the regions BC_LD-12, BC_DU-2 and BC_PI-7 contained 23, 9 and 12 SNPs respectively, segregating in all the backcrosses, therefore, the lack of SNP informativity was not the cause for the detection of backcross-specific QTL.

Moreover, in order to test the SNP number effect, an additional GWAS comparison within BC_DU using different SNPs number, employing 60K or 650K SNP chip genotyping information was conducted (results no shown). The QTL identification resulted similar between both platforms obtaining the same significant regions considering the different significance thresholds conditional on the tests number carried out in each analysis. The principal difference lies on the number of TAS within each region, being larger when employing the 650K, as expected, leading to a better definition of the QTL regions.

Candidate genes and SNPs

One of the major challenge in GWAS analysis, and in QTL scans in general, is the identification of candidate genes, and furthermore the identification of candidate causal mutations. The use of different approaches for this purpose, including functional information via data mining, gene expression data and SNP calling from RNA-Seq allowed us to identify potential candidate genes and promising SNPs. For common regions, a set of potential candidate genes is proposed, being the most relevant *CAST*, related to shoulder weight; *IL4R*, *NKX2-5*, *MYOD1* and *CNR1* associated with ham-weight and *MLH1* and *CAV1* related to intramuscular fat. Besides, candidate genes for backcross-specific QTL regions were identified, highlighting the *MUSK*, *SELL*/*SELP* and *HP* genes, associated with intramuscular fat, the *HADHA*/ *HADHB* genes related with shoulder-weight, the *IL2* gene associated with ham-weight, the *FAT1* gene associated with backfat thickness and the *CXCR4* gene for loin bone-in. Also promising SNPs were identified within these candidate genes, with interesting predicted effects such as: synonymous variants on *CAST* exon 26 and *CXCR4* exon 2, UTR variants on *CAV1*, *HP* and *SELL* genes, and missense variants on *IL4R* exon 9 and *MLH1* exon 9.

The design and analysis methodology employed in the current study, using

different genetic backgrounds and high density SNP data, has allowed us to determine 58 specific and 22 common QTL for growth, fatness and premier cut yield. Additionally, the integration of results from RNA-Seq assays and functional annotation has facilitated the selection of candidate genes and gene variants, providing a list of candidate mutations as *CAST, IL4R, CAV1, MLH1, HP, SELL, SELP* and *CXCR4* that need to be further investigated.

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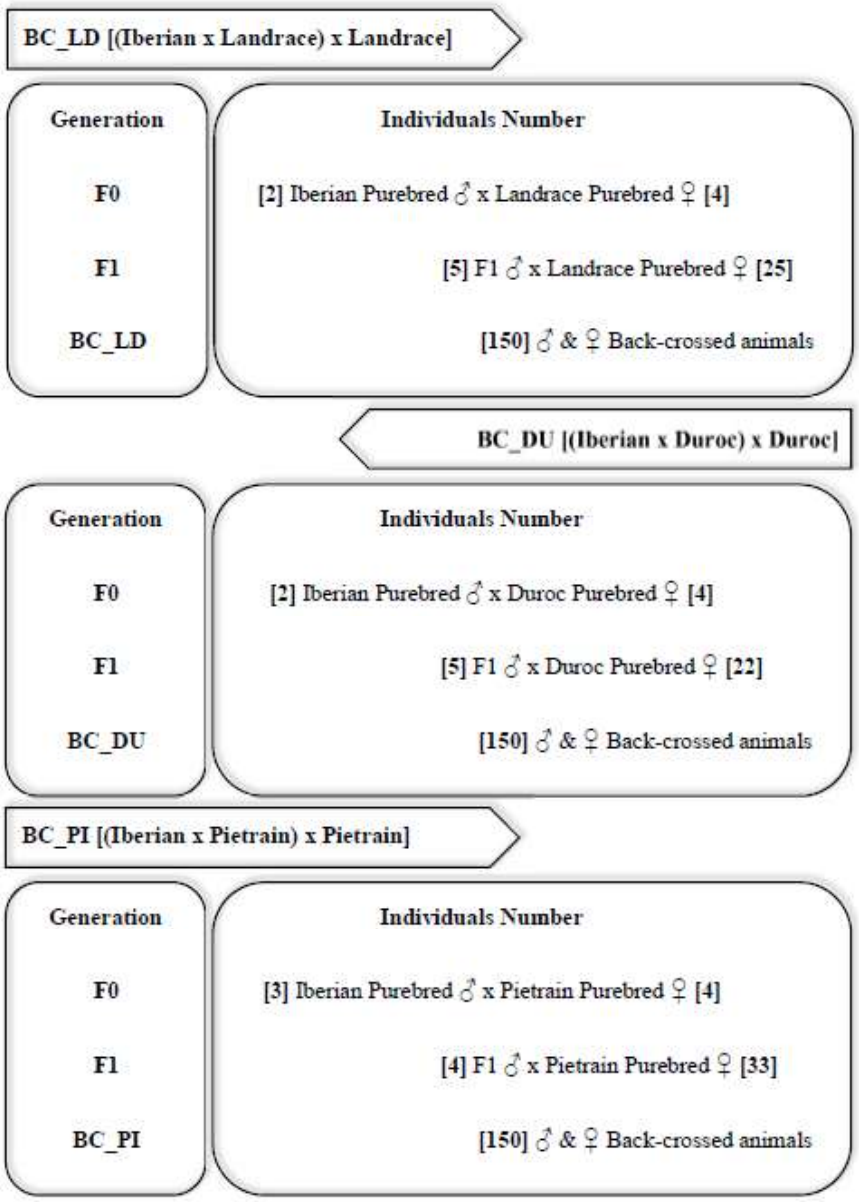

Fig 1: Backcross generation scheme: Schematic representation of each of the backcrosses used (BC_LD, BC_DU and BC_PI), specifying the number of individuals in each generation.

Table 1: Phenotypic traits recorded for the backcrossed pigs analyzed. Number of individuals (N), mean and standard deviation (SD) were calculated. Measures of body weight at 150 days of mean age (BW150), backfat thickness measured at 75kg (BFT75), weights of premium cuts, hams (HW) shoulders (SW) and loin bone-in (LBW) and intramuscular fat content (IMF) in *Longissimus dorsi* muscle.

	BW150	BFT75	HW	SW	LBW	IMF
Merged						
N	384	270	381	381	372	369
Mean	70.249	12.55	10.011	5.38	6.508	2.647
SD	12.09	2.511	1.322	0.746	1.012	1.341
BC_PI						
N	142	112	141	141	139	142
Mean	62.176	10.791	10.139	5.302	6.594	1.9
SD	10.616	1.639	1.293	0.728	1.031	0.748
BC_DU						
N	135	55	135	135	129	135
Mean	73.568	16.077	9.9	5.508	6.115	3.858
SD	10.105	1.721	1.326	0.725	0.865	1.324
BC_LD						
N	101	101	99	99	98	86
Mean	77.265	12.59	9.993	5.305	6.911	2.02
SD	10.068	1.437	1.35	0.786	0.995	0.631

Table 2: Number of TAS and regions identified in each GWAS (QTL) for the six different traits. Significance thresholds were estimated using QQ-Plot approach.

	MERGED		*BC_LD*		*BC_DU*		*BC_PI*	
	TAS	QTL	TAS	QTL	TAS	QTL	TAS	QTL
BW150	676	48	139	17	57	8	33	5
BFT75	26	2	102	17	195	25	20	4
HW	577	52	288	29	244	31	97	8
SW	48	5	193	17	119	15	14	4
LBW	14	2	816	49	171	21	122	2
IMF	449	38	84	9	72	10	327	30

Table 3: Common QTL regions: overlapping regions across independent backcross analyses (CR) and merged dataset (MR): genomic location, SNP count, associated trait and additive effects. The effect was estimated on the most significant marker of each QTL region. [1] Sum of SNPs count of each overlapping region.

Region	Genomic Position	SNPs Nº	Trait	a ± se
CR1	1:38,574,466:39,297,776	17 [1]	SW	0.10 ± 0.05
CR2	1:242,366,488:252,258,110	12 [1]	IMF	0,38 ± 0,17
CR3	2:4,872,634:12,620,747	68 [1]	HW	-0,37 ± 0,12
CR4	2:42,810,677:46,429,654	48 [1]	HW	0,29 ± 0,10
CR5	2:82,280,603:133,135,994	38 [1]	HW	0,37 ± 0,12
CR6	3:19,801,787:20,927,826	12 [1]	HW	0,25 ± 0,11
CR7	6:63,986,347:66,727,937	44 [1]	HW	-0,20 ± 0,09
CR8	6:136,192,384:142,925,060	23 [1]	HW	-0,30 ± 0,13
CR9	9:48,487,535:49,127,681	30 [1]	HW	0,32 ± 0,11
CR10	13:23,296,705:26,988,390	77 [1]	IMF	0,36 ± 0,09
CR11	13:79,798,036:91,100,617	10 [1]	HW	0.31 ± 0.11
CR12	13:150,479,945:151,823,774	19 [1]	LBW	-0,23 ± 0,07
CR13	15:19,138,505:27,279,315	103 [1]	HW	-0.26 ± 0.13
CR14	16:22,673,925:39,757,114	35 [1]	BW150	-3,63 ± 0,86
CR15	16:50,865,354:66,486,324	23 [1]	HW	-0,45 ± 0,14
MR1	1:49,725,381:50,914,824	2	BW150	3,93 ± 1,49
MR2	1:62,894,757:65,225,314	2	HW	0.35 ± 0.12
MR3	13:143,624,457:144,082,720	3	IMF	0,70 ± 0,20
MR4	13:150,711,607:150,723,061	2	SW	0.03 ± 0.01
MR5	13:180,877,890:183,593,430	4	HW	-0,24 ± 0,10
MR6	15:45,487,499:45,732,570	2	BW150	-2,37 ± 0,83
MR7	18:25,027,827:34,658,339	8	IMF	-0,83 ± 0,21

Table 4: Backcross F1 (Iberian x Landrace) x Landrace specific QTL regions: genomic location, SNP count, associated trait and additive affects. The effect was estimated on the most significant marker of each QTL region.

Region	Genomic Position	SNPs Nº	Trait	a ± se
BC_LD-1	1:3,282,730:6,036,055	13	BW150	5.18 ± 1.17
BC_LD-2	1:207,232,466:209,787,256	3	LBW	0.25 ± 0.08
BC_LD-3	1:229,999,129:230,057,074	4	LBW	0.23 ± 0.08
BC_LD-4	1:252,417,925:252,561,815	3	LBW	-0.27 ± 0.10
BC_LD-5	1:259,145,774:262,295,090	3	HW	-0.47 ± 0.11
BC_LD-6	1:281,527,840:284,386,253	2	IMF	-0.48 ± 0.16
BC_LD-7	1:292,340,489:308,184,301	16	LBW	-0.40 ± 0.13
BC_LD-8	2:157,223,320:157,336,158	4	LBW	0.24 ± 0.07
BC_LD-9	3:2,293,776:21,036,918	12	LBW	0.15 ± 0.07
BC_LD-10	3:18,811,772:19,921,287	4	LBW	0.18 ± 0.07
BC_LD-11	3:106,559,950:143,791,334	20	SW	-0.12 ± 0.04
BC_LD-12	4:60,516,616:71,501,440	23	LBW	0.24 ± 0.09
BC_LD-13	5:819,687:84,399,219	84	LBW	-0.29 ± 0.07
BC_LD-14	5:27,869,742:31,301,164	6	BFT75	-1.06 ± 0.33
BC_LD-15	5:84,355,028:84,502,147	3	HW	0.20 ± 0.09
BC_LD-16	6:116,675,908:121,495,633	5	HW	-0.20 ± 0.08
BC_LD-17	7:3,154,424:9,690,196	8	SW	-0.21 ± 0.06
BC_LD-18	7:123,169,296:128,857,481	5	LBW	-0.29 ± 0.10
BC_LD-19	8:39,395,095:40,884,155	5	BFT75	-0.79 ± 0.24
BC_LD-20	11:7,134,345:12,118,496	5	SW	-0.07 ± 0.035
BC_LD-21	11:20,049,479:20,304,315	4	BW150	5.01 ± 1.39
BC_LD-22	11:51,577,689:52,191,239	2	BFT75	0.98 ± 0.27
BC_LD-23	14:87,034,503:93,400,220	9	LBW	0.33 ± 0.13
BC_LD-24	14:141,402,773:143,979,213	4	HW	-0.18 ± 0.07
BC_LD-25	16:20,757,335:24,829,163	4	SW	-0.12 ± 0.04
BC_LD-26	16:57,488,206:57,543,891	2	SW	-0.15 ± 0.06
BC_LD-27	17:23,677,109:23,888,831	3	HW	-0.18 ± 0.07
BC_LD-28	17:37,474,260:37,724,038	3	IMF	0.32 ± 0.10
BC_LD-29	18:36,145,263:36,257,423	2	LBW	-0.21 ± 0.08

Table 5: Backcross F1 (Iberian x Duroc) x Duroc specific QTL regions: genomic location, SNP count, associated trait and additive affects. The effect was estimated on the most significant marker of each QTL region

Region	Genomic Position	SNPs Nº	Trait	a ± se
BC_DU-1	1:24,318,637:24,454,868	2	BFT75	-1.55 ± 0.55
BC_DU-2	1:105,063,134:112,726,301	9	HW	0.23 ± 0.08
BC_DU-3	2:365,309:2,525,121	2	BFT75	-0.88 ± 0.29
BC_DU-4	3:64,022,095:79,457,637	9	HW	-0.54 ± 0.12
BC_DU-5	4:33,037,772:33,879,368	3	SW	-0.12 ± 0.04
BC_DU-6	6:39,187,023:40,183,749	3	HW	-0.29 ± 0.13
BC_DU-7	7:134,055,500:134,540,651	7	BW150	4.46 ± 1.02
BC_DU-8	8:78,060,350:79,601,958	3	SW	-0.10 ± 0.04
BC_DU-9	8:108,681,177:111,104,427	6	SW	-0.16 ± 0.07
BC_DU-10	11:33,976,329:41,674,293	27	HW	-0.25 ± 0.07
BC_DU-11	17:8,862,441:9,863,762	2	BFT75	1.19 ± 0.35
BC_DU-12	18:5,935,981:6,138,538	2	BFT75	-1.64 ± 0.38

Table 6: Backcross F1 (Iberian x Pietrain) x Pietrain specific QTL regions: genomic location, SNP count, associated trait and additive affects. The effect was estimated on the most significant marker of each QTL region.

Region	Genomic Position	SNPs Nº	Trait	a ± se
BC_PI-1	2:19,707,886:36,428,198	26	IMF	-0.71 ± 0.17
BC_PI-2	2:87,305,602:89,995,349	3	LBW	0.19 ± 0.06
BC_PI-3	4:79,413,481:90,932,770	7	IMF	-0.47 ± 0.17
BC_PI-4	6:3,152,461:22,591,224	7	IMF	-0.54 ± 0.14
BC_PI-5	6:137,148,881:139,779,269	6	LBW	-0.24 ± 0.09
BC_PI-6	7:91,485,439:93,603,818	5	LBW	0.28 ± 0.09
BC_PI-7	8:130,390,887:146,084,493	12	IMF	0.26 ± 0.09
BC_PI-8	10:23,950,948:51,865,961	11	IMF	-0.24 ± 0.09
BC_PI-9	10:61,446,104:61,561,767	4	BW150	6.28 ± 1.56
BC_PI-10	11:50,946,880:51,225,411	2	LBW	-0.52 ± 0.10
BC_PI-11	11:80,613,144:84,900,189	3	LBW	-0.38 ± 0.11
BC_PI-12	12:1,431,153:8,877,446	12	IMF	-0.34 ± 0.09
BC_PI-13	14:129,849,565:132,601,975	7	LBW	-0.28 ± 0.07
BC_PI-14	15:13,648,821:20,934,106	5	LBW	-0.40 ± 0.08
BC_PI-15	15:57,805,024:62,049,028	4	LBW	0.28 ± 0.08
BC_PI-16	16:37,509,826:44,506,276	5	IMF	-0.43 ± 0.15
BC_PI-17	18:42,030,856:50,615,450	4	IMF	-0.46 ± 0.17

Table 7: Genomic location of the candidate genes identified in the common QTL regions. Functional candidates and differentially expressed (DE) in RNA-Seq analysis [37], [38].

	Chromosome	Gene Start	Gene End	Region
Functional Candidates				
CAST	2	106,934,106	107,128,389	CR5
ERAP1	2	107,133,359	107,167,934	CR5
PAM	2	112,397,803	112,553,942	CR5
IL4R	3	19,846,364	19,869,474	CR6
PGM1	6	137,171,271	137,233,581	CR8
ANGPTL3	6	138,101,691	138,111,290	CR8
NKX2-5	16	55,400,561	55,403,626	CR15
GSK3B	13	149,430,809	149,702,182	CR12
MLH1	13	23,613,451	23,692,645	CR10
SCN5A	13	25,518,200	25,604,929	CR10
SCN10A	13	25,662,389	25,791,413	CR10
MYOD1	2	44,482,283	44,485,063	CR4
KCNJ11	2	44,816,109	44,817,275	CR4
CNR1	1	63,202,833	63,204,251	MR2
CAV1	18	31,745,664	31,780,340	MR7
PPP1R3A	18	34,430,772	34,468,824	MR7
ING3	18	27,551,031	27,581,190	MR7
DE Candidates				
DAB2	16	25,736,189	25,907,473	CR14
EGFLAM	16	24,682,029	24,902,072	CR14
CCNT2	15	19,431,039	19,473,796	CR13
CENPH	16	51,246,712	51,272,145	CR15
DUSP1	16	55,768,409	55,771,513	CR15
UPK1B	13	150,294,290	150,320,054	CR12
DCLK3	13	23,956,907	23,989,416	CR10

Table 8: Genomic location of the candidate genes identified in the backcross F1 (Iberian x Landrace) x Landrace specific QTL regions. Functional candidates and differentially expressed (DE) in RNA-Seq analysis [37], [38].

	Chromosome	Gene Start	Gene End	Region
Functional Candidates				
MUSK	1	282,424,654	282,563,435	BC_LD-6
PTGS1	1	294,753,878	294,780,705	BC_LD-7
ENG	1	302,232,063	302,266,135	BC_LD-7
CRAT	1	303,403,630	303,417,444	BC_LD-7
ASS1	1	304,454,129	304,508,998	BC_LD-7
ABL1	1	304,685,889	304,829,672	BC_LD-7
ACTB	3	4,729,276	4,732,654	BC_LD-9
GTF2IRD1	3	11,276,910	11,374,748	BC_LD-9
PHKG1	3	17,074,784	17,082,851	BC_LD-9
MYLPF	3	18,376,798	18,379,771	BC_LD-9
ATP2A1	3	18,733,279	18,753,571	BC_LD-9
EIF2AK2	3	109,622,369	109,667,785	BC_LD-11
HADHB	3	119,649,281	119,690,928	BC_LD-11
HADHA	3	119,690,599	119,782,862	BC_LD-11
POMC	3	120,766,301	120,772,980	BC_LD-11
ACP1	3	142,383,410	142,396,888	BC_LD-11
PEX2	4	64,727,532	64,745,314	BC_LD-12
JPH1	4	67,243,406	67,423,310	BC_LD-12
WNT1	5	15,421,207	15,424,241	BC_LD-13
IGFBP6	5	18,768,002	18,772,057	BC_LD-13
SP1	5	19,024,788	19,065,851	BC_LD-13
KRAS	5	52,229,480	52,271,842	BC_LD-13
IAPP	5	55,323,600	55,326,804	BC_LD-13
LRP6	5	63,577,474	63,674,469	BC_LD-13
TNFRSF1A	5	66,780,726	66,793,255	BC_LD-13
ADIPOR2	5	70,999,571	71,021,751	BC_LD-13
VDR	5	81,277,766	81,339,276	BC_LD-13
EDN1	7	9,152,653	9,159,251	BC_LD-17
DICER1	7	123,586,576	123,624,391	BC_LD-18
VRK1	7	125,267,394	125,310,472	BC_LD-18
KL	11	9,345,005	9,392,395	BC_LD-20
SUCLA2	11	20,069,061	20,123,486	BC_LD-21
SFTPD	14	88,660,304	88,675,023	BC_LD-23
HTRA1	14	143,497,174	143,558,530	BC_LD-24
AMACR	16	20,765,284	20,782,822	BC_LD-25
PRLR	16	21,539,826	21,558,491	BC_LD-25

DE Candidates				
ADAMTS13	1	306,998,075	307,029,937	BC_LD-7
PCOLCE	3	7,918,398	7,923,583	BC_LD-9
KRT8	5	18,663,339	18,671,193	BC_LD-13
LYZ	5	36,179,190	36,185,575	BC_LD-13
MFAP5	5	65,575,791	65,589,682	BC_LD-13
FGF23	5	68,250,561	68,261,760	BC_LD-13
WNK1	5	70,264,406	70,410,707	BC_LD-13
ANO6	5	79,126,662	79,217,840	BC_LD-13
DSP	7	4,968,322	5,021,221	BC_LD-17
SH2D4B	14	88,988,037	89,072,555	BC_LD-23
AGXT2	16	21,478,635	21,519,015	BC_LD-25
EGFLAM	16	24,682,029	24,902,072	BC_LD-25

Table 9: Genomic location of the functional candidate genes identified in the backcross F1 (Iberian x Duroc) x Duroc specific QTL regions.

	Chromosome	Gene Start	Gene End	Region
Functional Candidates				
SMAD2	1	106,901,465	106,991,088	BC_DU-2
SMAD7	1	108,087,684	108,117,509	BC_DU-2
MYO5B	1	109,020,804	109,331,777	BC_DU-2
SMAD4	1	110,509,117	110,563,187	BC_DU-2
FGF3	2	1,965,237	1,973,613	BC_DU-3
FGF4	2	2,004,484	2,006,245	BC_DU-3
FGF19	2	2,060,948	2,064,674	BC_DU-3
HK2	3	71,331,772	71,407,448	BC_DU-4
ACTG2	3	72,251,856	72,310,387	BC_DU-4
DYSF	3	74,465,104	74,674,249	BC_DU-4
SCN1B	6	40,022,675	40,031,626	BC_DU-6
LRAT	8	78,710,098	78,717,666	BC_DU-8
IL2	8	108,797,155	108,802,321	BC_DU-9
FAT1	17	9,339,790	9,455,277	BC_DU-11

Table 10: Genomic location of the candidate genes identified in the backcross F1 (Iberian x Pietrain) x Pietrain specific QTL regions. Functional candidates and differentially expressed (DE) in RNA-Seq analyses [37], [38].

	Chromosome	Gene Start	Gene End	Region
Functional candidates				
LYN	4	82,710,960	82,765,981	BC_PI-3
SNAI2	4	86,680,287	86,682,763	BC_PI-3
PRKDC	4	87,185,966	87,343,303	BC_PI-3
SELE	4	88,914,284	88,924,075	BC_PI-3
SELL	4	88,931,478	88,951,561	BC_PI-3
SELP	4	88,990,349	89,025,954	BC_PI-3
KARS	6	11,900,776	11,914,646	BC_PI-4
FA2H	6	12,797,985	12,853,562	BC_PI-4
TAT	6	14,064,568	14,076,437	BC_PI-4
HP	6	14,643,378	14,648,241	BC_PI-4
ITGB1	10	61,450,695	61,502,941	BC_PI-9
CXCR4	15	18,124,941	18,128,996	BC_PI-14
ELOVL7	16	42,502,362	42,590,592	BC_PI-16
GHRHR	18	46,427,127	46,436,822	BC_PI-17
DE Candidates				
ANO3	2	36,225,783	36,430,350	BC_PI-1
IBSP	8	140,438,440	140,452,173	BC_PI-7
C1QL3	10	50,066,724	50,073,357	BC_PI-8
NPTX1	12	1,938,599	1,944,323	BC_PI-12
GALR2	12	5,264,762	5,267,427	BC_PI-12

Table 11: Most promising SNPs identified in the current study: SNPs selected by gene function and allele frequencies within the backcrossed pigs analysed by RNA-Seq: Gene name, reference allele, alternative allele, chromosome, position, predicted effect and alternative allele frequencies.

Gene	Ref	Alt[A1]	Chr	Position	Effect prediction	Alt Freq
CAST	T	C	2	107,114,719	synonymous variant	0.84
IL4R	A	T	3	19,848,238	missense variant	0.71
CAV1	A	G	18	31,746,137	3 prime UTR variant	0.78
CAV1	G	A	18	31,746,065	3 prime UTR variant	0.40
MLH1	C	G	13	23,653,815	missense variant	0.38
MLH1	A	G	13	23,653,910	missense variant	0.49
HP	G	A	6	14,644,715	5 prime UTR variant	0.29
SELL	G	A	4	88,931,558	5 prime UTR variant	0.25
SELL	T	C	4	88,950,415	3 prime UTR variant	0.25
SELL	A	G	4	88,950,439	3 prime UTR variant	0.29
CXCR4	C	G	15	18,127,836	synonymous variant	0.38

SUPPORTING INFORMATION CAPTIONS

S1 Figure: Graphical representation of the minimum allele frequency (MAF) of the SNPs included in each of the datasets analyzed (BC_LD, BC_DI, BC_DU and Merged dataset).

S2 Figure: QQ-Plots for each one of the GWAS analysis carried out with each dataset (BC_LD, BC_DI, BC_DU and Merged dataset) and the phenotypic traits BW150, BFT75, HW, SW, LBW and IMF.

S3 Figure: Manhattan Plots for each one of the GWAS analysis carried out with each dataset (BC_LD, BC_DI, BC_DU and Merged dataset) and the phenotypic traits BW150, BFT75, HW, SW, LBW and IMF. Significance thresholds were estimated using QQ-Plot approach.

S4 Table: Candidate SNPs identified for the candidate genes selected in the common regions, backcross-specific regions. Genomic location, reference allele (Ref) and alternative allele (Alt).

S5 Table: VeP effect prediction for selected candidate SNPs

Discusión

El objetivo de la presente tesis doctoral ha sido la caracterización de las bases genéticas de la regulación del crecimiento, deposición grasa y rendimiento de piezas nobles en porcino, implementando y explorando las posibilidades que ofrecen las tecnologías de análisis y secuenciación masiva. El análisis global de los datos obtenidos, mediante la integración de diversas aproximaciones, ha permitido la obtención de un amplio abanico de resultados que van desde la identificación de QTL a potentes genes y mutaciones candidatos (Figura 9). Todos estos resultados abren las puertas a una gran variedad de estudios específicos.

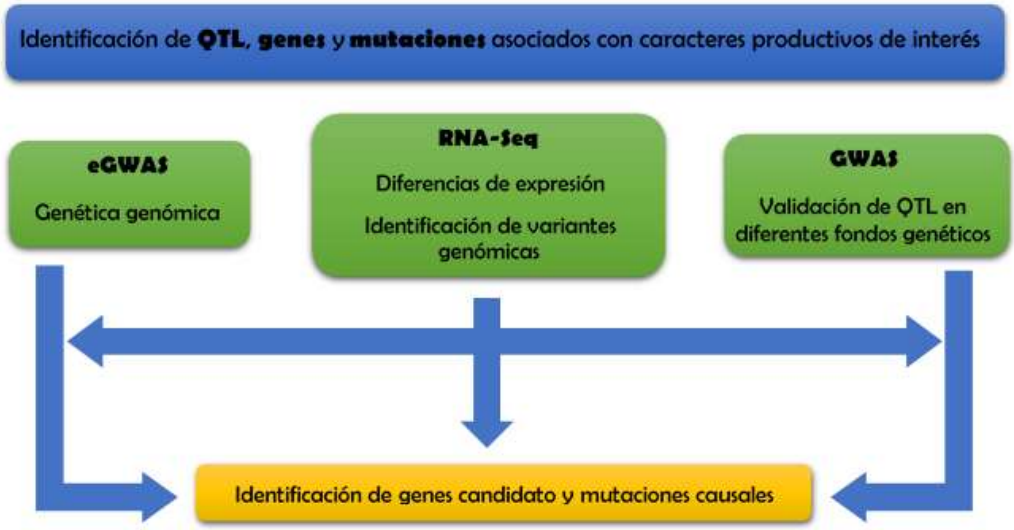

Figura 9: Gráfica de integración de los diferentes estudios realizados para la identificación de regiones del genoma, genes candidato y potenciales mutaciones causales asociadas con crecimiento, deposición grasa y rendimiento de piezas nobles

La utilización de la tecnología de RNA-Seq es una de las aproximaciones más empleadas en la actualidad para el estudio del transcriptoma. Además de las mejoras implícitas al hecho de analizar niveles de expresión a nivel de genoma completo, existen otras ventajas asociadas con el diseño de esta plataforma basada en secuenciación, como es la identificación de nuevos tránscritos (Chen *et al*. 2011; Pérez-Montarelo *et al*. 2014; Puig-Oliveras *et al*. 2014; Muñoz *et al*. 2017) o la identificación de nuevas variantes génicas. En el primero de los trabajos presentados en esta tesis doctoral se ha demostrado la viabilidad del RNA-Seq aplicado a la identificación de variantes génicas asociadas con caracteres productivos a partir de un diseño experimental basado en la utilización de grupos extremos para caracteres de crecimiento y deposición grasa (Grupos A y B),

determinados mediante un análisis de componentes principales, de individuos del retrocruce F1 (Ibérico x Landrace) x Landrace. Mediante esta técnica se han podido identificar más de 90.000 SNPs localizados en transcritos expresados en hígado e hipotálamo, que aportan una gran cantidad de información para ser utilizada en estudios posteriores. Además de la identificación de variantes génicas, se seleccionaron aquellos SNPs que fuesen más informativos relacionados específicamente con los caracteres productivos de interés, seleccionando aquellos en homocigosis o heterocigosis en uno de los grupos y que presentaban el genotipo alternativo en el otro, pudiéndose identificar 4.396 y 1.862 SNPs informativos en hipotálamo e hígado respectivamente. A partir de estos SNPs informativos se identificaron posibles mutaciones causales en genes propuestos como candidatos para la regulación de deposición grasa y crecimiento, seleccionados por función y por localización en QTL previamente descritos en el material IBMAP, concretamente en los genes *RETSAT*, *RNMT* y *PALMD.* La validación de la capacidad del RNA-Seq como método para identificación de variantes génicas, se llevó a cabo utilizando el método tradicional de secuenciación Sanger, panel de 32 SNPs, pudiéndose identificar una tasa de falsos positivos (FDR) del 20%. Este estudio muestra que la tecnología de RNA-Seq es una herramienta útil para identificar variantes génicas, pero pone en evidencia algunos factores que pueden afectar a la fiabilidad de los resultados. Entre ellos cabe destacar que en los estudios basados en el transcriptoma se permiten valores de cobertura altos para poder capturar transcritos con niveles de expresión elevados, lo que puede provocar un aumento del FDR, lo que no sucede en las técnicas basadas en secuenciación del genoma donde se establecen valores de cobertura máximos para evitar falsos positivos. A su vez, hay que tener en cuenta que existe una tasa de error de asignación de nucleótidos, dependiente de la plataforma de secuenciación utilizada, siendo en este caso Illumina, que muestra tasas de entre el 0,3% y 3,8% al inicio y fin de las lecturas respectivamente (Dohm *et al*. 2008). Cabe destacar que las herramientas de análisis de este tipo de datos, tanto de filtrado, como de alineamiento y de identificación de SNPs establecen criterios modificables que permiten ser más estrictos o laxos modificando así tanto la tasa de falsos positivos como de falsos negativos. Por lo tanto, aunque esta tecnología es muy sólida para la identificación de SNPs, se debe optimizar modificando los criterios de identificación de variantes, teniendo en cuenta los posibles errores en la secuenciación, en el filtrado y en el alineamiento.

El estudio de genética genómica llevado a cabo en este trabajo, mediante la integración de medidas de expresión correlacionados con caracteres para crecimiento y deposición grasa, denominados fenotipos intermedios, junto a técnicas de análisis de asociación de genoma completo (eGWAS) ha permitido obtener resultados interesantes que ayudan a descifrar la regulación de los genes involucrados en crecimiento y deposición grasa. En primer lugar, los resultados del análisis eGWAS han permitido identificar 954 asociaciones significativas entre 42 genes y 880 eTAS (SNPs asociados con fenotipos de expresión génica). A partir de estas asociaciones, se pudieron identificar 63 regiones genómicas o eQTL, localizadas en todos los autosomas menos SSC10, SSC12, SSC16 y SSC18, poniendo de manifiesto la complejidad de la regulación de la expresión génica. Los resultados obtenidos en este segundo trabajo aportan una gran cantidad de información de eQTL que contienen potenciales mutaciones causales para la regulación de genes relacionados con crecimiento y deposición grasa. La eficacia de esta aproximación se puede confirmar con la validación de la relación entre el gen *IGF2* y eTAS localizados en las regiones SSC2:16.416–10.979.357 y SSC2:162.084.552–162.298.086, donde previamente se ha descrito la mutación causal que afecta a la expresión de *IGF2* en músculo, relacionado con desarrollo muscular y composición de ácidos grasos (Van Laere *et al*. 2003; Hong *et al*. 2015).

Es necesario resaltar que la gran cantidad de datos manejados en este estudio, datos de expresión génica de miles de sondas contenidas en el chip de expresión porcino y miles de SNPs del chip de 60K porcino, requiere de un pre-procesado eficaz para poder llevar a cabo un análisis refinado y coherente, es por ello que en este trabajo se realizó un filtrado inicial de los datos de expresión, determinándose un umbral de correlación mínima entre las sondas de expresión y los caracteres analizados. Los esfuerzos en la interpretación y posteriores análisis se centraron en potenciales genes y SNPs candidatos localizados en regiones eQTL que solapasen con QTL previamente descritos en el mismo material. Aunque con esta aproximación algunos de los resultados del estudio se quedan sin analizar, sirviendo de base para posteriores estudios, se identificaron 6 eQTL que solapan con QTL descritos en SSC4 para deposición grasa (Varona *et al*. 2002; Fernández *et al*. 2012) y en SSC9, SSC13 y SSC17 para rendimiento de piezas nobles y deposición grasa (Fernández *et al*. 2012). Más aún, la integración de los eQTL identificados en este trabajo junto a los resultados obtenidos de la identificación de SNPs mediante RNA-Seq ha permitido la identificación de SNPs informativos y que además se localizan en genes

localizados dentro de los eQTL, pudiendo proponerse 20 potenciales mutaciones causales localizadas en los genes *ZNF786*, *ACAD11*, *RYK*, *MGLL*, *TRIB3*, *PDIA4*, *LAMB1*, *RBP1*, *TXNRD3* e *ICA*. El efecto de estos SNPs sobre la expresión génica se validó mediante estudios de asociación, postulándose nueve de estos como claros candidatos a ser mutación causal reguladora, o al menos estar localizados cerca de ella, en los genes *MGLL, ICA, RYK, TXNRD3, RBP1* y *TRIB3*. Por otro lado, se evaluó el efecto de estos SNPs sobre los caracteres productivos estudiados, identificándose efectos claros en los SNPs *MGLLg*.88218G>A, *TXNRD3g*.25947T>A, *TXNRD3g*.25928C>T y *RYKg*.75088C>T con los caracteres de peso a los 150 días de edad y rendimiento de piezas nobles.

Uno de los resultados más interesantes de este trabajo correspondió a la identificación de un ARN largo no codificante (ARNlnc), codificado por el gen *ALDBSSCG0000001928*, cuya expresión esta correlacionada con los caracteres analizados, localizado a menos de 3 kb del gen *TXNRD3* y a menos de 600 kb del gen *MGLL*, genes donde se localizaron SNPs asociados con los cambios de expresión de *ALDBSSCG0000001928*. Además, se determinó una correlación negativa entre los niveles de expresión de los genes *MGLL* y *TXNRD3* con la expresión de *ALDBSSCG0000001928*. Teniendo en cuenta todos estos resultados se propuso un mecanismo de regulación del polimorfismo *TXNRD3g*.25928C>T afectando a caracteres productivos, a través de la regulación de la expresión de un ARNlnc, que a su vez estaría regulando la expresión de genes adyacentes mediante represión transcripcional.

Aunque este tipo de estudios permite la identificación de una gran cantidad de regiones QTL asociadas con caracteres productivos, la identificación de los genes y mutaciones causales (QTNs) sigue siendo todavía un reto que se enfrenta a diferentes limitaciones. Entre las más significativas cabría destacar las asociadas con el número de individuos analizados y la validación de esos QTL en diferentes fondos genéticos (Würschum and Kraft 2014; Kristensen *et al*. 2015; Van Eenennaam *et al*. 2014; Ashton *et al*. 2016). Para intentar solventar esta limitación se diseñó un estudio mediante GWAS en el que se incorporó información fenotípica y genotípica de diferentes retrocruces basados en cerdo Ibérica con algunas de las razas comerciales más relevantes, Landrace, Duroc y Pietrain. El objetivo de este estudio se centró en la validación de regiones QTL en diferentes fondos genéticos, así como la evaluación del efecto del número de individuos analizados. Para ello se realizaron GWAS independientes para cada uno de los

retrocruces y un GWAS conjunto en el que se incorporaron todos los datos de los retrocruces. Los resultados de asociación han permitido identificar más de 3.600 SNPs asociados a los seis caracteres productivos analizados, generando una base de información muy importante para estudios posteriores. A partir de estas asociaciones, se determinaron 458 regiones QTL, pudiéndose detectar QTL comunes entre distintos fondos genéticos, objetivo principal de este trabajo, y regiones QTL cruce-específicos, proporcionando información relevante asociada con el fondo genético. Así se determinaron 22 regiones QTL comunes, 15 identificadas a partir de los análisis independientes y coincidentes en al menos dos de los retrocruces analizados y en el análisis del material fusionado y 7 regiones identificadas únicamente con el material fusionado. Este resultado demuestra la mayor potencia obtenida al incluir un mayor número de animales, que, aun teniendo diferentes fondos genéticos, han permitido la identificación de QTL no detectados en los análisis individuales. A su vez, se pudieron identificar un total de 58 QTL específicos de retrocruce, coherentes con las características específicas de cada una de las tres razas empleadas en cada uno de los retrocuces.

Asimismo, se estudió el efecto del número de SNPs incorporados en los análisis GWAS. Esto se hizo en el retrocruce F1 (Ibérico x Duroc) x Duroc, donde se disponía de datos de genotipado de más de 650.000 SNPs genotipados con la plataforma Axiom® Porcine Genotyping Array. Para ello se llevó a cabo un estudio comparativo de los resultados del GWAS incluyendo todos los SNPs o sólo los SNPs equivalentes a la plataforma PorcineSNP60 BeadChip. Los resultados de este análisis mostraron un aumento significativo en el número de TAS al utilizar un mayor número de SNPs, diez veces más. Sin embargo, en cuanto al número de regiones QTL significativas identificadas, se obtuvieron resultados similares en ambos análisis (Figura 10). Estos resultados parecen indicar que la incorporación de más SNPs al estudio no aporta información significativa en la identificación de regiones asociadas, claramente condicionada por el número de recombinaciones existentes, pero sí parece incrementar en algunos casos la precisión en la detección de dichas regiones, por lo que al plantearse la selección de la plataforma de genotipado a usar en un estudio de asociación de genomas completos habría que sopesar el coste frente al beneficio que supone el uso de una mayor cantidad de marcadores.

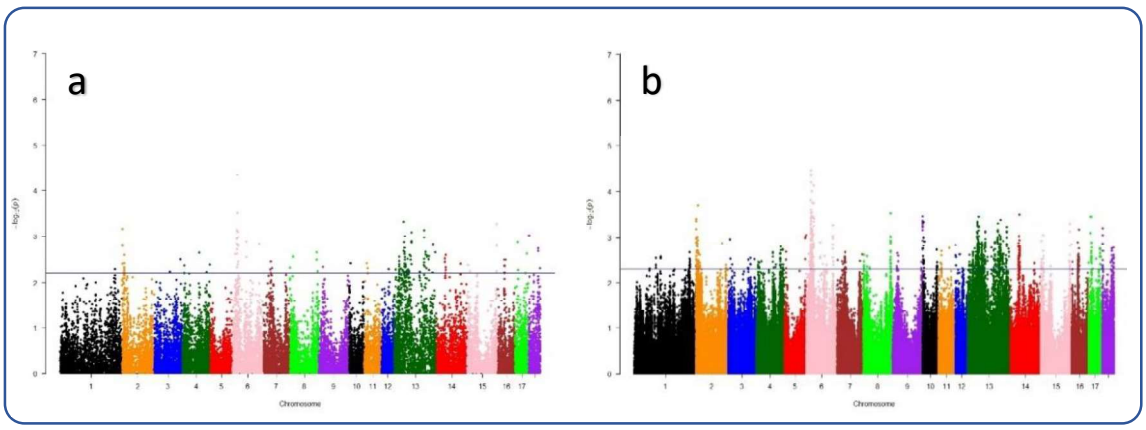

Figura 10: Manhattan plots del GWAS para peso del chuletero: a) 38.684 SNPs equivalentes a los contenidos en el chip PorcineSNP60BeadChip, b) 533.708 SNPs del chip Axiom Porcine de 650K.

Además de intentar superar las limitaciones asociadas al número de individuos en el diseño experimental y la validación de QTL en distintos fondos genéticos, también se ha llevado a cabo una integración de datos de diferentes tecnologías de análisis masivos. A través del uso de herramientas bioinformáticas para la caracterización funcional de los genes localizados en los QTL, se seleccionaron un set de genes candidatos para la regulación de crecimiento y deposición grasa, siendo los más interesantes: *HADHA*, *HADHB, MYOD1, NKX2-5, SCN1B, IL2, FAT1, IL4R, CAST, MLH1, MUSK, SELL, SELP, HP, CXCR4, CAV1* y *CNR1*. Para identificar potenciales mutaciones causales se llevó a cabo un planteamiento similar al del estudio anterior de eGWAS, empleando resultados de identificación de SNPs de datos procedentes de un ensayo de RNA-Seq en tejido muscular, hepático e hipotalámico en los tres retrocruces. Del total de SNPs identificados en los tres retrocruces, 1.425.878 SNPs, se seleccionaron 104 SNPs prometedores como mutaciones causales candidatas que cumplen diferentes propiedades, estando localizadas en QTL relevantes y genes candidatos por su función o por presentar niveles de expresión diferencial entre retrocruces, además de encontrarse segregando en todos los retrocruces, para los QTL comunes, o solamente en uno de los retrocruces para los QTL específicos.

Más aún, la comparación de los dos estudios de GWAS, para caracteres fenotípicos y de expresión, ha permitido detectar regiones QTL asociadas con expresión y con crecimiento, deposición grasa y rendimiento de piezas nobles comunes entre ambos estudios. Para ello se ha realizado una comparación de la posición de dichos QTL, así

como de los caracteres con los que se asocian. Se han podido identificar 39 regiones genómicas solapando entre ambos estudios (Tabla 4), 21 eQTL con 18 QTL. Estas regiones suponen claros candidatos sobre los que centrar esfuerzos en estudios posteriores para identificar potenciales reguladores de la expresión de genes asociados con crecimiento y deposición grasa, destacando los eQTL R4 y R10 que solapan con las regiones QTL BC_LD-7, BC_LD-9 y BC_LD-10 localizados en los cromosomas SSC1 (292-309 Mb) y SSC2 (14-21 Mb) respectivamente.

Tabla 4: Regiones QTL solapadas entre los estudios de genética genómica y de validación en diferentes fondos genéticos para los caracteres Peso a los 150 días (P150D), Espesor de tocino dorsal a 75 kg (ETD75), Peso medio de Jamones (JAM), Paletas (PAL) y Chuletero (CHU).

eQTL			**QTL**	
Región	Sonda de expresión	Carácter	Región	Carácter
R1	Ssc.1860.1.S1_at	JAM/PAL	BC_LD-1	P150D
R4	Ssc.30633.1.S1_at	CHU	BC_LD-7	CHU
R5	Ssc.9365.2.S1_a_at	P150D/JAM/PAL/CHU	CR3	JAM
R6	Ssc.20525.1.S1_at	JAM/PAL	CR3	JAM
R7	Ssc.10952.1.S1_at	P150D	CR5	JAM
R8	Ssc.20525.1.S1_at	JAM/PAL	BC_LD-8	CHU
R9	Ssc.9365.2.S1_a_at	P150D/JAM/PAL/CHU	BC_LD-8	CHU
R10	Ssc.29388.1.A1_at	CHU	BC_LD-9	CHU
R10	Ssc.29388.1.A1_at	CHU	BC_LD-10	CHU
R13	Ssc.26316.1.S1_at	ETD75/JAM/PAL/CHU	BC_LD-11	PAL
R18	Ssc.9109.1.A1_at	ETD75/JAM/PAL/CHU	BC_LD-12	CHU
R20	Ssc.1790.1.S1_at	JAM/PAL/CHU	BC_LD-13	CHU
R30	Ssc.15678.1.A1_s_at	P150D/JAM/PAL/CHU	BC_LD-18	CHU
R31	Ssc.21543.1.S1_at	JAM/PAL	BC_LD-18	CHU
R36	Ssc.9916.1.S1_at	ETD75/JAM/PAL/CHU	BC_LD-21	P150D
R40	Ssc.24997.1.S1_at	ETD75	CR10	GIM
R49	Ssc.10589.1.A1_at	P150D/JAM/PAL/CHU	CR12	CHU
R49	Ssc.10589.1.A1_at	P150D/JAM/PAL/CHU	MR4	PAL
R52	Ssc.30987.1.S1_at	JAM/PAL/CHU	BC_LD-24	JAM
R56	Ssc.7666.1.A1_at	P150D	BC_LD-27	JAM
R57	Ssc.21242.1.S1_at	ETD75/CHU	BC_LD-27	JAM
R61	Ssc.21242.1.S1_at	ETD75/CHU	BC_LD-28	GIM

El uso de herramientas bioinformáticas para anotar la funcionalidad de los genes y la predicción del efecto las variantes génicas es clave para llegar a proponer mutaciones

candidata con mayor fiabilidad, ya que la validación funcional del gen y mutación causal es un paso requerido y necesario para demostrar finalmente la causalidad de cualquier mutación causal identificada. En este trabajo se han empleado diversas herramientas como VarElect, FatiGO y STRING para examinar la funcionalidad de los genes candidato propuestos (*SYVN1*, *LARS*, *RETSAT*, *COPA*, *CADM3*, *EFNA1*, *PALMD*, *CNN3*, *MKL1*, *RHBDL2*, *JAK1*, *HAO1*, *RNMT*, *SERPINE1*, ZNF786, *ACAD11*, *RYK*, *MGLL*, *TRIB3*, *PDIA4*, *LAMB1*, *RBP1*, *TXNRD3*, *ICA*, *HADHA*, *HADHB*, *MYOD1*, *NKX2*-5, *SCN1B*, *IL2*, *FAT1*, *IL4R*, *CAST*, *MLH1*, *MUSK*, *SELL*, *SELP*, *HP*, *CXCR4*, *CAV1* y *CNR1)*. Adicionalmente, se han realizados análisis de interacción *in silico* entre genes utilizando las herramientas GeneMania (genemania.org), que permite establecer relaciones entre genes identificados mediante distintas aproximaciones (Figura 11).

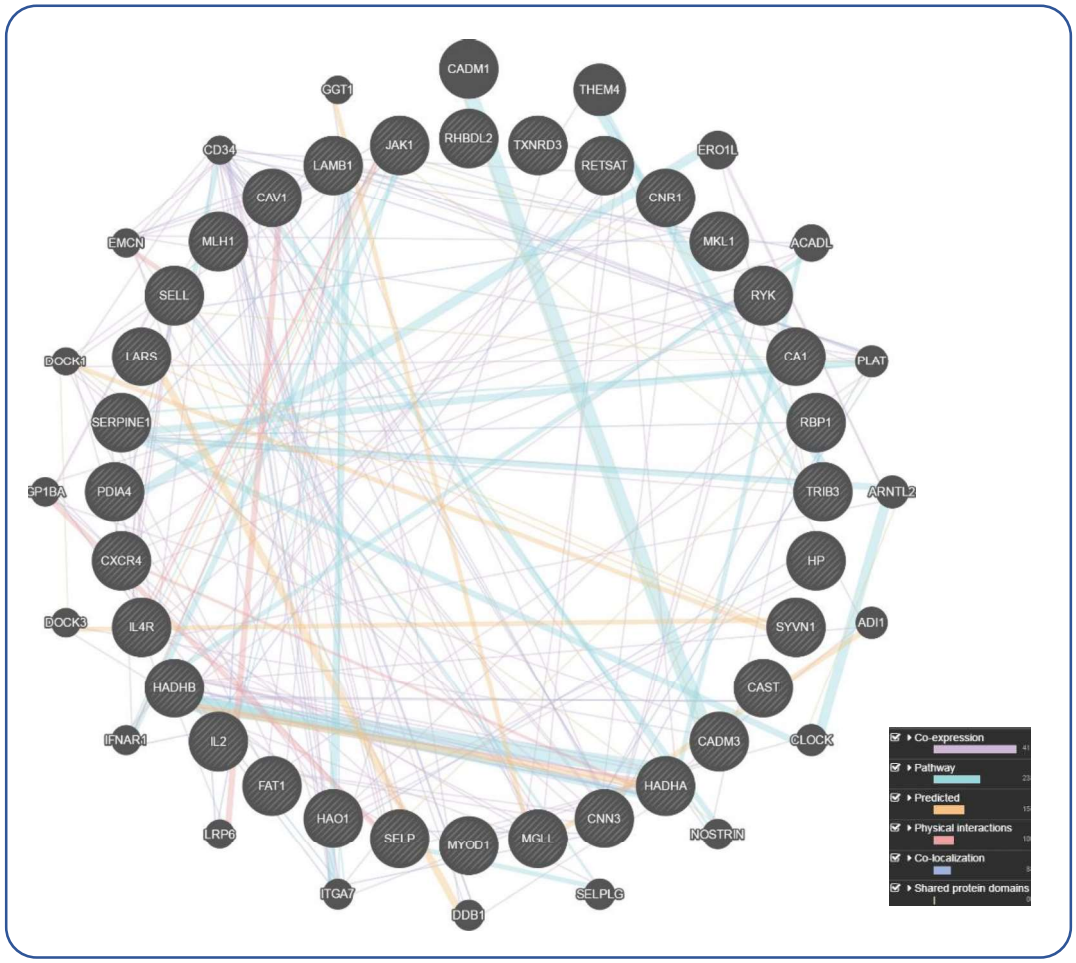

Figura 11: Red génica construida mediante GeneMania de los 41 genes seleccionados como candidatos potenciales en la presente tesis doctoral, y potencialmente involucrados en crecimiento y deposición grasa

Siendo las relaciones más interesantes las siguientes:

Interacción *SYVN1, PDIA4*, *HADHA* y *HADHB*. El gen *SYVN1*, identificado en el estudio de identificación de variantes mediante RNA-Seq, codifica una proteína involucrada en el control de calidad proteico del retículo endoplásmico a través de la degradación asociada al retículo endoplásmico (ERAD), habiendo sido descrita su intervención en procesos relacionados con el gasto energético en tejido adiposo (Fujita *et al*. 2015) y con la respuesta a estrés en miocitos (Doroudgar *et al*. 2015). *PDIA4*, identificado en el estudio de genética genómica, ha sido relacionada con la actividad de la chaperona *HSP90* en el proceso de plegamiento proteico y desarrollo muscular en musculo esquelético (García de la Serrana and Johnston, 2013). Los genes *HADHA* y *HADHB*, identificados en el estudio GWAS comparativo, han sido previamente relacionados con el aumento de la grasa intramuscular y disminución en la tasa de crecimiento (Sudre *et al*. 2005), además de mostrar cambios de expresión en musculo esquelético, asociado con la tasa de crecimiento en cerdos Landrace (Komatsu *et al*. 2016). La integración de esta información permite proponer la interacción de estos genes para posteriores estudios, como posibles candidatos para la regulación del desarrollo muscular y de tejido adiposo.

Interacción *RHBDL2* y *MLH1*: El gen *RHBDL2*, obtenido en el estudio de identificación de variantes mediante RNA-Seq, ha sido relacionado con la mediación en el proceso de diferenciación de adipocitos a través de la liberación de factores de crecimiento solubles (Pascall and Brown 2004; Harrington *et al*. 2007). *MLH1* codifica para una proteína de reparación de ADN, en el cual se ha podido identificar polimorfismos asociados con caracteres productivos relacionados con deposición grasa en Holstein (Lee *et al*. 2016). El estudio de la posible interacción entre estos dos genes podría facilitar información sobre la regulación de los caracteres asociados con deposición grasa en porcino.

Interacción *JAK1, IL2* e *IL4R*: La interacción entre los genes *JAK1*, obtenido en el estudio de identificación de variantes mediante RNA-Seq, y los genes *IL2* e *IL4R*, identificados en el estudio GWAS comparativo, ha sido previamente descrita relacionada con el efecto de la Leptina, miogénesis, crecimiento y la lipolisis de adipocitos, a través de la ruta génica *JAK/STAT* (Zieba *et al*. 2005; Pijet *et al*. 2013; Li *et al*. 2010; Arrizabalaga *et al*. 2012). La identificación de la relación entre estos genes obtenidos a partir de diferentes tecnologías permite tanto validar este tipo de aproximaciones en la

identificación de las bases genéticas de caracteres productivos, así como apoyar la importancia de la ruta *JAK/STAT* en la regulación del crecimiento y la deposición grasa en porcino.

Además, se han utilizado en este trabajo diferentes herramientas bioinformáticas para la predicción de los efectos de mutaciones génicas como *Variant effect predictor VeP* de ensembl para determinar la localización génica de SNPs identificados, así como predecir el posible efecto de dichas variantes a nivel de ADN, RegRNA para identificar el efecto de las mutaciones sinónimas a nivel de ARN y PredictProtein, SNAP2 y SIFT para predecir el efecto de cambio de amino acido en la secuencia proteica. Las mutaciones más relevantes identificadas en estos trabajos se pueden agrupar según los efectos predichos por dichas herramientas en diferentes categorías: Cambio de aminoácido tolerado: *RETSAT*:g.3320A>C, *ZNF786g*.6991A>G, *ACAD11g*.52909A>G, *ICAg*.28150G>C, *COG3*:g.4525A>G y *ACSM2B*:g.13374T>A. Posible efecto sobre eventos de splicing: *TXNRD3g*.25746T>C, *RYKg*.75088C>T, *RETSAT*:g.3320A>C, *COG3*:g.4525A>G y *ACSM2B*:g.13374A>T. Posible efecto sobre dianas de miRNAs: *ZNF786g*.8083C>T, *RETSAT*:g.3661delinsT y *NR3C1*:g.102797T>C. Estos polimorfismos componen una lista de potenciales mutaciones causales asociadas con caracteres productivos.

Finalmente, como resultados más novedosos extraídos en este trabajo destacan la identificación de modificaciones post-transcripcionales de edición del ARN y la detección de un ARNlnc implicado en la regulación de caracteres productivos.

Edición de ARN:

Durante el proceso de identificación de SNPs mediante RNA-Seq, se obtuvieron algunos resultados llamativos en algunos SNPs localizados en los genes *NR3C1*, *COG3* y *ACSM2B*, al identificar genotipos diferentes en los distintos tejidos analizados de un mismo individuo, lo que podría estar asociado con expresión alélica especifica de tejido. Sin embargo, la validación de estos SNPs mediante secuenciación de Sanger en el mismo material de estudio, así como en muestras de hipotálamo de seis animales Ibéricos puros y cruce Ibérico x Large White, mostraron resultados sorprendentes. Algunos tránscritos mostraron alelos que no correspondían con la información contenida en la secuencia de ADN genómico, confirmando que se trataba de fenómenos de edición de ARN. La utilización de información bibliográfica y la utilización de herramientas bioinformáticas

permitieron identificar *in silico*, potenciales implicaciones funcionales de estas tres modificaciones, como aparece reflejado en el primero de los estudios.

ARN largo no codificante:

Como se ha comentado anteriormente, en el estudio de genética genómica se identificó un RNA largo no codificante, codificado por el gen *ALDBSSCG0000001928*, candidato a regular la variabilidad de los caracteres estudiados. En la literatura se puede encontrar la relevancia que están adquiriendo este tipo de elementos como reguladores de la expresión génica tanto a nivel transcripcional como post-transcripcional, en el procesado de ARN mensajeros y sus posibles mecanismos de acción (Angrand *et al*. 2015) (Figura 12).

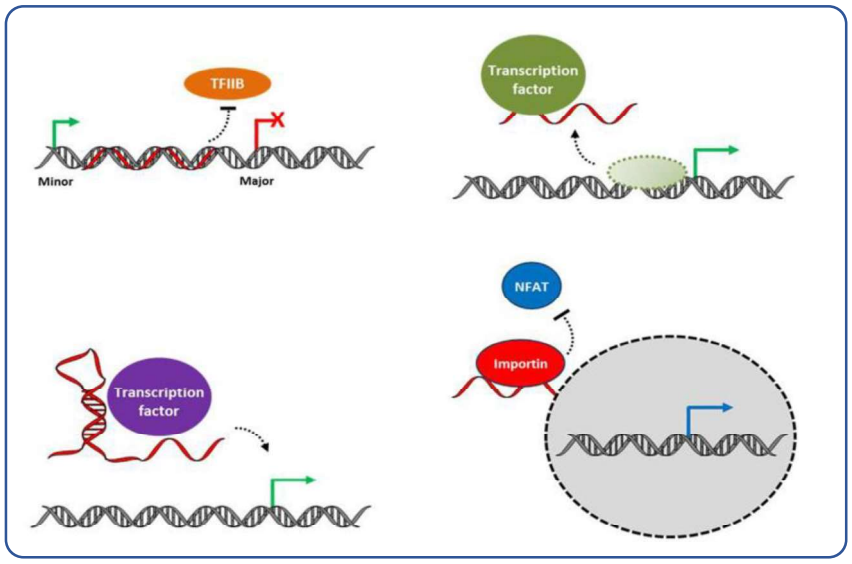

Figura 12: Esquema representativo de los posibles mecanismos de acción de los elementos ARNlnc para regular

La caracterización y los mecanismos de acción de los miRNAs sobre la regulación de la expresión génica lleva tiempo siendo estudiada tanto en humanos como en diferentes especies animales (Gulyaeva and Kushlinskiy, 2016), e incluso se han establecido asociaciones entre diferentes miRNAs y caracteres productivos relacionados con el metabolismo energético y desarrollo muscular en diferentes especies ganaderas (Guo *et al*. 2014; Wang *et al*. 2014). Sin embargo, no se trata del único mecanismo para la regulación de la expresión génica mediada por ARN no codificante, sino que se ha podido identificar asociación de elementos ARNlnc con caracteres productivos mediada por cambios en la regulación génica, en particular en la especie porcina (Weikard *et al*. 2015).

Estos resultados confirman la eficacia de este tipo de estudios en la búsqueda de variaciones genéticas asociadas a caracteres de interés, pudiéndose identificar mecanismos novedosos de regulación que están adquiriendo una mayor relevancia en investigación.

Posibles futuras aplicaciones:

Los resultados obtenidos en este trabajo conforman una base sólida para la identificación de genes y mutaciones causales de la variabilidad de los caracteres productivos porcinos analizados. Sin embargo, hay que remarcar que estos resultados no son directamente aplicables en programas de selección, ya que requieren estudios que permitan establecer la relación de causalidad de los genes y las mutaciones propuestas. Por tanto, a partir de estos resultados, se pueden plantear múltiples estudios futuros que vayan encaminados hacia esta dirección como:

- Diseño de paneles de genotipado de media densidad conteniendo los polimorfismos candidatos propuestos para llevar a cabo validaciones genéticas de los efectos detectados en poblaciones comerciales.
- Genotipado de las variantes identificadas que sufren edición de RNA sobre transcritos en tejido hepático e hipotalámico, por considerarse como los principales tejidos encargados del metabolismo energético, homeostasis y control del apetito, y asociación con caracteres productivos.

Conclusiones

Los estudios realizados bajo el marco de esta tesis doctoral han dado lugar a gran cantidad de información y resultados que incrementan el conocimiento sobre la base genética de caracteres productivos en porcino y sobre aspectos metodológicos más generales que se pueden englobar en las siguientes conclusiones:

1. La tecnología de secuenciación del RNA, RNA-Seq, permite la identificación fiable de polimorfismos en tránscritos expresados que pueden ser explotados como fuente de información en múltiples análisis. Además, con un apropiado diseño y análisis de datos, permiten identificar posibles mutaciones causales.

2. La edición del ARN es un fenómeno nada despreciable que puede ser responsable de la variabilidad de caracteres de interés. Este fenómeno afecta a la tasa de falsos negativos en la validación de polimorfismos identificados mediante RNA-Seq.

3. El estudio de asociación de genomas completos llevado a cabo con datos de expresión de genes relacionados con los caracteres analizados, ha permitido poner de manifiesto la complejidad de la regulación génica, identificando más de 60 eQTL. Sin embargo, su integración con resultados de QTL para caracteres fenotípicos potencia la identificación de regiones asociadas, genes e incluso mutaciones candidatas a regular la variabilidad de dichos caracteres.

4. La integración de genotipos y fenotipos de tres fondos genéticos distintos en análisis de asociación de genomas completos ha permitido identificar 22 regiones QTL comunes, incrementando probablemente el éxito en la identificación de QTNs. Por otro lado, los resultados ponen de manifiesto la relevancia de QTL específicos, 58 QTL retrocruce-específicos, que muestran efectos concordantes con los fondos genéticos analizados.

5. La integración de los resultados de los dos últimos estudios permite destacar 18 regiones QTL asociadas a caracteres de deposición grasa y rendimiento de piezas nobles. Dichas regiones han sido identificadas de manera independiente en cada una de las aproximaciones, potenciando su valor.

6. Fruto de los estudios llevados a cabo se propone una serie de genes y mutaciones candidatas cuya relevancia viene soportada por análisis *in silico* con diversas herramientas bioinformáticas. Entre los genes candidato propuestos, destaca la identificación del gen *ALDBSSCG0000001928* asociado con caracteres de

crecimiento y rendimiento, que corresponde a un ARN largo no codificante (ARNlnc), elementos recientemente descritos como clave en la regulación génica.

7. La gran cantidad de información y resultados obtenidos serán útiles para estudios futuros sobre éste y otro material animal. Además de justificar el planteamiento de estudios más específicos enfocados al análisis de los genes y mutaciones candidatas propuestas.

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Anexo

MATERIAL SUPLEMENTARIO

Table S1 Primers and amplification conditions used for SNP validation, Sanger sequencing on cDNA and gDNA, pirosequecing and qPCR.

SNP identification	NCBI accession number	Gene symbol	Primers Fw/Rv	Fragment size	Tª an.
ENSSSCG00000027057g.2895G>T	ss1985400201	*SYVN1*	GGTGACGGGTGCCAAGAGACT/GGGGGCCAGAGCGGGAACAT	268	66
ENSSSCG00000014401g.102797T>C	ss1985401074	*NR3C1*	AGAACTGGCAACGCTTTTATCAA/TGCCCCCAAGTTATTATTTCTCA	471	54
ENSSSCG00000014411g.33280A>C	ss1985400202	*LARS*	TGTTGCAGGGAGGTGAC/TAAAGGAAATATGAAAGATGAT	236	51
ENSSSCG00000014411g.7010G>A	ss1985400203	*LARS*	CCAGATGAAGAAGAGGAAGAG/CAGCGGTGGGAAATACT	211	53
ENSSSCG00000007858g.13374T>A	ss1985401075	*ACSM2B*	AGACCTGCAAGCCACTGACATAA/GTAGTAGCAACATCCGGTAAACAA	216	57
ENSSSCG00000008237g.3320A>C	ss1985400207	*RETSAT*	CACCGAGGGGCAACTGGACTGG/GGTGGATGCGCGGAGGAACG	302	62
ENSSSCG00000006386g48255C>T; g..48429G>T; g.48430G>T	ss1985400208	*COPA*	TGAGAAGAACCCCACAGATGC/GTGGGAGGGGAAGAGATAAATACT	597	TDown62
ENSSSCG00000006415g.21844T>C	ss1985401078	*CADM3*	CAAGTGCCAAGTGAAAGAGC/CAGTTGAGGGTGGTTGTATCC	314	58
ENSSSCG00000006530g.5633T>C	ss1985400210	*EFNA1*	GCCCATGCCAATCCACAG/TGACAGCGCCACAGAGG	689	TDown62
ENSSSCG00000006582g.1877C>T	ss1985401079	*S100A14*	TCGGTGGAGGGTGGGAAGGAGA/TTGGGTCGGGGAAGAGATGAGGAG	659	TDown62
ENSSSCG00000006727g.49406C>G	ss1985401080	*WDR3*	GCTTGTGCCAGTGATTGAAAAA/AATGGAGCCCGTACTATGTTGG	383	TDown62
ENSSSCG00000006874g.210G>C	ss1985400211	*PALMD*	TGTGCCGCTGCCGTTCAA/GGTGCCTCAGTTTTTCTTCCTCTA	396	TDown62
ENSSSCG00000006887g.17250A>G	ss1985400212	*CNN3*	AGCCAAGTGCTAGGAACCTC/TCGTTCACCTTCTTCACGGAG	283	62
ENSSSCG00000000075g.10685T>C	ss1985400213	*MKL1*	GAGCTGAAGCCAAAGGTGAAGAAG/GCTGGCGCTGCTGCTGGTG	274	TDown74
ENSSSCG00000028272g.48226T>C	ss1985401081	*FP565371.2*	ACTCCGTTTAACAGACAGCAATGG/AAACTCGCCCGATCCTTACCT	583	59
ENSSSCG00000030076g.27047A>G	ss1985401082	*SLC6A13*	CCCTGGGCAGCTACAACAAGT/CCAACCCCAGGAGGACGACCAT	277	62
ENSSSCG00000003247g.4656C>T	ss1985401083	non-annotated	CAGGTGTGGTGGCCGTGTATGTAG/GGGTATGGGGCCAGGAAGGAG	333	TDown72
ENSSSCG00000003651g.19048A>G	ss1985400214	*RHBDL2*	AGTTCTGCCTGGGACACC/AGGGGGCGGGAGGCAGTTA	274	TDown70
ENSSSCG00000003809g.18273A>G	ss1985401084	*JAK1*	GAGACCCCAAGACGGAGCAG/TGAAATCAGTAACATAGACG	364	52
ENSSSCG00000003809g.12288A>G	ss1985400215	*JAK1*	TACGCTCCGAATCGCACCATC/GGGAGCCAGGCATTTGACCA	253	58
ENSSSCG00000023489g.4832C>A	ss1985401085	*CXCL9*	AAAATTGATTAAAGAGTGGGAGAA/GTGGGATGTGTTTGTGAGGATG	437	53
ENSSSCG00000010884g.14128A>G	ss1985401086	non-annotated	GTTTCCCTCCGTGGTAGTTTC/TGGGACAGGCTTCAGATG	400	60
ENSSSCG00000027815g.4525A>G	ss1985401087	*COG3*	GTTCAAGGGATTATCACAGGAG/AAAAATCTTGGAACAGTCATAGG	250	53
ENSSSCG00000017383g.4388C>A	ss1985401088	*AOC3*	GGGGCTACCGCATCCAG/CCTCCTGCAGTCAACTGA	235	59
ENSSSCG00000010487g.9565G>A	ss1985401089	non-annotated	CCGGCGCTTCTCCCTCAT/TTTTCATTTAATTTTTCCAACAAG	241	59
ENSSSCG00000010488g.39756G>A	ss1985401090	non-annotated	CAAGAATTCCCCAACCCAGAGG/CAAAGCCCAGAAGAGGACGAGTTA	346	57
ENSSSCG00000010739g.20840C>G	ss1985401091	*CTBP2*	CACGCAGGAGATCCACGAGAAGGT/CCGCCGGTACAGGTTGAGGATG	255	TDown62
ENSSSCG00000027439g.7569A>T	ss1985400216	*HAO1*	CTCCGGAATGTGGCTGAAGTAGAC/GTAAAGTTGCAGCCAGCGAAGTG	238	57
ENSSSCG00000025855g.185A>C	ss1985400217	*LOC100626038*	CCGGGACCAGGCTTTCT/AATTCGACTTTGGCTACGCTTCT	396	59
ENSSSCG00000025698g.1249A>G	ss1985400218	*SERPINE1*	ATGGGGCCGTGGAACAAAGATGA/TGTGGAAGAGGCGGTGGTGAGTG	326	62
RNA-editing					
SNP validation on gDNA					
ENSSSCG00000014401g.102797T>C	ss1985401074	*NR3C1*	AGAACTGGCAACGCTTTTATCAA/TGCCCCCAAGTTATTATTTCTCA	1000	54
ENSSSCG00000007858g.13374T>A	ss1985401075	*ACSM2B*	CCCCCACCCCCAACACA/CTTCCTGAACACTCGCCATCCAT	377	62
ENSSSCG00000027815g.4525A>G	ss1985401087	*COG3*	AAGGGGAGCAGGTGACAGAGTTAG/GTGCCAGCCAGGACAATGA	360	56
Pyrosequencing on cDNA					
ENSSSCG00000027815g.4525A>G	ss1985401087	*COG3*	CGCTCCATTTCACACTGAATT/CAGCCTAAAAAATCTTGGAACAG/CTGAATTCACCATTAAGGA	115	53
Pyrosequencing on gDNA					
ENSSSCG00000027815g.4525A>G	ss1985401087	*COG3*	ATAATTGTAACGTGGGAATATGGG/GCCAGGACAATGAAGTACCTCTA/TGAATTCACCATTAAGGAA	302	53
SNP Genotyping for assocaition analyses					
ENSSSCG00000008237g.3320A>C	ss1985400207	*RETSAT*	CACCGAGGGGCAACTGGACTGG/GGGTGGATGCGCGGAGGAAC	951	61
ENSSSCG00000000075g.10685T>C	ss1985400213	*MKL1*	GAGCTGAAGCCAAAGGTGAAGAAG/GCTGGCGCTGCTGCTGGTG	370	TDown74
ENSSSCG00000025698g.1249A>G	ss1985400218	*SERPINE1*	CCCCTGCCCTCCGTCAACTGTA/AATGCCGGATCCTTAACCCACTG	525	54

ARTÍCULO 2: Deciphering the regulation of porcine genes influencing growth, fatness and yield-related traits through genetical genomics.

Supplemental Table 1: Primers designed for the genotyping of TXNRD3, PDIA4, TRIB3, and MGLL genes

ss2031475809	
Fw-biotin	B-5´AGCAGGACAATGACTTTCAAACT3´
Rv	5´TCGGTGTTTTCAAGATCAAATTAG3´
Seq	5´GTCTGTGTGTGCATGTT3´
ss2031475808	
Fw	B-5´AGCAGGACAATGACTTTCAAACT3´
Rv	5´TCGGTGTTTTCAAGATCAAATTAG3´
Seq	5´GTCTGTGTGTGCATGTT3´
ss2031475804	
Fw	5´GTTCGTTTCGCGAGACCAG3´
Rv	B-5´CCGCTCACCTTCGTCTGG3´
Seq	5´CCTGGCTCAGCTGCTGGCCG3´
ss2031475807	
Fw	5'CCGCCCTGCCTCTCCCAAAAG3'
Rv	5'GCAGGGGGCTTCCAGGACTGAG3'
ss2031475817	
Fw	5'GGAGTTCCTGTTGTGGCTCA3'
Rv	5'GTTGAATCCGCCAGACCTGA3'

ARTÍCULO 2: Deciphering the regulation of porcine genes influencing growth, fatness and yield-related traits through genetical genomics.

Supplemental Table 2: Correlation among probeset expression and phenotypic traits.

Probeset	Trait	Correlation	P-val
AFFX.Ss_18SrRNA_at	IMF	0.350826119	0.000322029
RPTR.Ssc.AF292559.4_s_at	BFS	-0.341673812	0.000440418
RPTR.Ssc.AJ002682.1_s_at	BF75	-0.334992583	0.000578363
RPTR.Ssc.M15077.1_s_at	BFS	-0.37131627	0.000121935
Ssc.10000.1.A1_at	HW	-0.367752857	0.000180581
Ssc.10013.1.A1_at	BF75	-0.350413112	0.000305529
Ssc.10047.1.A1_at	BW150	0.348102024	0.000414489
Ssc.10051.2.A1_at	BF75	-0.321559477	0.000982286
Ssc.10169.1.A1_at	HW	-0.371786657	0.000151249
Ssc.10226.2.A1_at	BFS	-0.355630028	0.000244365
Ssc.10259.1.A1_at	BF75	-0.448639399	2.25544E-06
Ssc.10298.1.A1_at	BW150	-0.370341113	0.000161211
Ssc.10312.1.A1_at	BFS	-0.342735755	0.000421514
Ssc.10316.1.S1_at	LBW	0.339453581	0.000587585
Ssc.10407.1.A1_at	BF75	-0.391208158	4.79132E-05
Ssc.10431.1.A1_at	BF75	-0.345098465	0.000382102
Ssc.10504.1.A1_at	BF75	-0.362757124	0.000178972
Ssc.10532.1.A1_at	BFS	-0.427481129	7.42357E-06
Ssc.10589.1.A1_at	HW	-0.363068023	0.000221216
Ssc.10595.1.A1_at	BFS	-0.325516552	0.000842471
Ssc.10618.1.A1_at	HW	-0.337584417	0.000632788
Ssc.1062.1.A1_at	BF75	-0.367727533	0.000143408
Ssc.10656.1.A1_at	HW	-0.330102517	0.000847436
Ssc.10657.1.A1_at	HW	-0.335112383	0.000697462
Ssc.10679.1.S1_at	HW	-0.352514328	0.000345545
Ssc.10733.1.A1_at	BF75	-0.34432432	0.000394625
Ssc.10743.1.A1_at	BW150	-0.52498274	2.42762E-08
Ssc.10745.1.A1_at	BF75	-0.366748539	0.000149847
Ssc.10785.1.A1_at	BFS	-0.333836715	0.000605907
Ssc.10791.1.A1_at	BFS	-0.328873457	0.000738382
Ssc.10826.1.A1_at	BFS	-0.407321615	2.14908E-05
Ssc.10860.1.S1_at	BF75	-0.421869804	1.00484E-05
Ssc.10864.1.A1_at	HW	-0.452292777	2.60363E-06
Ssc.10952.1.S1_at	BW150	-0.351528579	0.000359961
Ssc.11057.3.S1_at	BF75	-0.353437104	0.000268548
Ssc.11075.11.S1_x_at	IMF	-0.444904294	3.14072E-06
Ssc.11158.2.S1_at	HW	-0.341260078	0.000546728
Ssc.11208.1.S1_at	HW	0.331672125	0.000797551
Ssc.11224.1.S1_at	HW	0.350393145	0.000377251
Ssc.11284.1.A1_at	BW150	-0.341528321	0.000540888
Ssc.11375.1.A1_at	BF75	-0.402933793	2.68447E-05
Ssc.11393.1.A1_at	BFS	-0.340355862	0.000464962
Ssc.11487.1.A1_at	BF75	-0.369989611	0.000129499
Ssc.11494.1.A1_at	BF75	-0.341467615	0.000444177
Ssc.11563.1.S1_at	BW150	-0.329585917	0.000864468
Ssc.11696.1.A1_at	BF75	-0.3644721	0.000165869
Ssc.11704.1.A1_at	BFS	-0.378782951	8.64783E-05
Ssc.11779.1.S1_at	HW	0.342306735	0.000524265
Ssc.1180.1.S1_at	BF75	0.321414338	0.000987795
Ssc.11812.1.A1_at	BW150	-0.479920134	4.99585E-07
Ssc.11828.1.A1_at	BF75	-0.50592165	5.82194E-08
Ssc.11867.1.A1_at	BFS	-0.360268881	0.000199694
Ssc.11890.1.A1_at	BF75	-0.466917788	7.54178E-07
Ssc.11896.1.A1_at	BFS	-0.337783568	0.000516521
Ssc.11906.1.S1_at	HW	0.395226494	5.1518E-05
Ssc.11907.1.A1_at	BF75	-0.402894221	2.68982E-05
Ssc.11924.1.A1_at	BF75	-0.323919039	0.000896568

Ssc.11990.1.S1_at	BF75	-0.481776278	2.95048E-07
Ssc.11994.1.A1_at	BFS	-0.334028357	0.000601258
Ssc.12.1.S1_at	IMF	-0.335969824	0.000592908
Ssc.1200.1.S1_at	LBW	-0.33599333	0.000673752
Ssc.12015.1.A1_at	BF75	-0.479611538	3.39216E-07
Ssc.12022.1.S1_at	HW	0.340183335	0.000570755
Ssc.12031.1.A1_at	BF75	-0.355828913	0.000242275
Ssc.12032.1.S1_at	HW	0.349000175	0.000399506
Ssc.12053.1.A1_at	BF75	-0.335423811	0.000568384
Ssc.121.1.S1_at	BF75	-0.567973734	4.79119E-10
Ssc.12174.1.A1_at	BF75	-0.358262605	0.000217996
Ssc.12181.1.A1_at	BF75	-0.504684947	6.34605E-08
Ssc.12316.2.A1_at	BF75	-0.386712847	5.94875E-05
Ssc.12335.2.S1_a_at	HW	0.3504099	0.000376991
Ssc.12363.1.A1_at	BFS	-0.350715966	0.000301625
Ssc.12379.1.A1_at	BF75	-0.417719505	1.25271E-05
Ssc.1238.1.S1_at	HW	0.362224971	0.000229369
Ssc.12408.1.A1_at	BF75	-0.429921395	6.49677E-06
Ssc.12419.1.A1_at	BFS	-0.321440648	0.000986795
Ssc.12421.1.A1_at	HW	-0.344845733	0.000473246
Ssc.12428.1.A1_at	BFS	-0.338832173	0.0004949
Ssc.12459.1.A1_at	BF75	-0.374236856	0.000106707
Ssc.125.1.S1_at	BF75	-0.510758626	4.14205E-08
Ssc.1250.1.A1_at	HW	-0.335936792	0.000675251
Ssc.12540.1.A1_at	BW150	-0.407855586	2.78597E-05
Ssc.12605.1.A1_at	BFS	-0.345096711	0.00038213
Ssc.1269.1.A1_at	BW150	-0.57672112	4.17414E-10
Ssc.12702.1.A1_at	BFS	-0.329990924	0.000706434
Ssc.12745.1.A1_at	BF75	-0.407530703	2.12625E-05
Ssc.1281.1.S1_at	HW	0.36226999	0.000228927
Ssc.12887.1.A1_at	HW	-0.357202701	0.000283979
Ssc.12893.1.A1_at	HW	-0.33015389	0.000845759
Ssc.129.1.S1_at	BF75	-0.324345753	0.000881816
Ssc.12902.1.A1_at	BF75	-0.436059582	4.62402E-06
Ssc.12941.3.S1_a_at	BW150	-0.327265067	0.000944904
Ssc.1303.2.A1_at	BW150	-0.405648081	3.10759E-05
Ssc.13092.1.A1_at	BF75	-0.453389994	1.70694E-06
Ssc.13115.1.A1_at	BF75	-0.323250894	0.00092012
Ssc.13160.1.A1_at	BF75	-0.360181622	0.000200459
Ssc.1317.1.S1_at	LBW	0.32897015	0.000885176
Ssc.13175.1.A1_at	BFS	-0.359711715	0.000204629
Ssc.13176.3.S1_at	BF75	-0.500733147	8.33949E-08
Ssc.13179.1.A1_at	HW	-0.368272305	0.000176529
Ssc.13195.1.A1_at	BFS	-0.327483811	0.000779955
Ssc.13214.1.A1_at	BF75	-0.37694165	9.41981E-05
Ssc.13227.1.A1_at	BF75	-0.336516854	0.000543795
Ssc.13256.1.A1_at	BF75	-0.336554545	0.000542965
Ssc.13326.2.A1_at	BFS	-0.342675263	0.000422571
Ssc.1333.1.A1_at	BW150	-0.381006109	9.99834E-05
Ssc.13430.1.A1_at	BW150	-0.326991453	0.000954824
Ssc.13454.1.A1_at	BF75	-0.403463072	2.61383E-05
Ssc.13479.1.A1_at	BF75	-0.344862845	0.000385875
Ssc.13500.1.A1_at	BW150	-0.370154609	0.00016254
Ssc.13502.1.S1_at	BF75	-0.322746818	0.00093826
Ssc.13504.1.A1_at	BFS	-0.339945374	0.000472859
Ssc.13568.1.A1_at	HW	-0.440151878	5.14474E-06
Ssc.13585.7.S1_a_at	HW	-0.329492832	0.00086757
Ssc.13585.8.S1_x_at	HW	-0.345885444	0.000453703
Ssc.13644.1.A1_at	LBW	-0.328089415	0.000915583
Ssc.13769.1.S1_at	BF75	-0.410286887	1.84585E-05
Ssc.13773.1.S1_at	BW150	-0.388042654	7.22903E-05
Ssc.13862.2.S1_a_at	HW	0.337030455	0.000646784
Ssc.13877.1.A1_at	HW	-0.352114236	0.000351331
Ssc.13884.1.A1_at	BW150	-0.37579735	0.000126517
Ssc.1391.1.S1_at	BF75	-0.323436652	0.000913516
Ssc.1392.1.S1_at	BF75	-0.345178181	0.000380834

Ssc.13938.1.S1_at	IMF	-0.368197807	0.0001517
Ssc.14081.1.A1_at	BFS	-0.361856802	0.000186228
Ssc.14173.1.S1_at	BW150	-0.341665049	0.000537934
Ssc.14185.1.A1_at	BF75	-0.358048674	0.000220036
Ssc.14187.1.A1_at	BW150	-0.32599684	0.000991687
Ssc.14343.1.S1_at	BF75	0.376896166	9.43967E-05
Ssc.14431.1.S1_at	BFS	0.396341754	3.72793E-05
Ssc.14463.1.S1_at	BFS	0.339560919	0.000480367
Ssc.14479.1.S1_at	IMF	-0.323589992	0.000963955
Ssc.1455.1.A1_at	BF75	-0.416542619	1.33282E-05
Ssc.14551.1.S1_at	BF75	-0.388913515	5.35295E-05
Ssc.15146.1.S1_at	HW	0.366604512	0.000189846
Ssc.1524.1.S1_at	BFS	-0.38304737	7.08024E-05
Ssc.15266.2.S1_at	BW150	-0.363170722	0.000220241
Ssc.15394.1.S1_at	HW	-0.334792448	0.000706261
Ssc.15433.1.S1_at	BFS	-0.407379894	2.1427E-05
Ssc.15435.1.S1_at	BF75	-0.324195585	0.000886982
Ssc.15592.1.S1_at	BW150	0.337741147	0.000628879
Ssc.15650.2.A1_at	HW	0.339268687	0.00059192
Ssc.15665.1.A1_at	BW150	-0.333030151	0.000756571
Ssc.15665.1.S1_at	BF75	-0.329702482	0.000714557
Ssc.15678.1.A1_s_at	HW	-0.356011432	0.000298582
Ssc.15740.2.A1_at	BF75	-0.461598702	1.04418E-06
Ssc.15777.1.S1_at	BF75	-0.416699215	1.32189E-05
Ssc.1579.1.S1_at	HW	0.334956663	0.000701732
Ssc.15819.1.A1_at	BF75	-0.326278489	0.000817734
Ssc.15852.1.S1_x_at	BF75	-0.352551713	0.000278924
Ssc.15860.1.A1_s_at	BF75	-0.342994246	0.000417027
Ssc.15910.1.A1_at	BFS	-0.43795644	4.15717E-06
Ssc.15911.1.A1_at	BFS	-0.344616574	0.000389854
Ssc.15923.1.A1_at	BFS	-0.35447194	0.000256874
Ssc.15939.1.S1_at	BF75	-0.539792904	4.78187E-09
Ssc.15966.1.S1_at	BF75	0.33255826	0.000637768
Ssc.15984.2.S1_at	BFS	-0.429585915	6.61738E-06
Ssc.16016.1.S1_at	BFS	-0.422999644	9.45826E-06
Ssc.16034.1.A1_at	BF75	-0.424278875	8.82948E-06
Ssc.16050.1.A1_at	BF75	-0.370698041	0.000125408
Ssc.16066.1.S1_at	BFS	-0.361074343	0.000192755
Ssc.16072.1.A1_at	BF75	-0.360364525	0.000198858
Ssc.16074.1.A1_at	HW	-0.333797723	0.000734268
Ssc.16100.1.A1_at	BFS	-0.377798218	9.05304E-05
Ssc.16101.1.S1_at	BW150	-0.468793996	9.88351E-07
Ssc.16105.1.S1_at	IMF	-0.332150804	0.000690301
Ssc.1615.2.A1_at	HW	-0.427567797	1.01434E-05
Ssc.16192.1.S1_at	BFS	-0.417425014	1.27232E-05
Ssc.16204.1.A1_at	BF75	-0.401927655	2.82371E-05
Ssc.16297.1.S2_at	BFS	-0.341749272	0.000439049
Ssc.1630.1.S1_at	IMF	-0.357237388	0.000245136
Ssc.16313.1.S1_at	IMF	-0.331081046	0.000720094
Ssc.16320.1.A1_at	BFS	-0.338730002	0.00049697
Ssc.16353.1.S1_at	BW150	-0.476107562	6.3288E-07
Ssc.16377.1.A1_at	IMF	-0.330682856	0.000731481
Ssc.16390.3.A1_at	BF75	-0.392681295	4.4603E-05
Ssc.16410.1.A1_at	IMF	-0.378650419	9.44416E-05
Ssc.16622.1.A1_at	BF75	-0.38983079	5.12145E-05
Ssc.16626.1.A1_at	BF75	-0.352361803	0.000281197
Ssc.16645.1.S1_at	LBW	-0.338399791	0.000612691
Ssc.16787.1.A1_at	BFS	-0.360642418	0.000196448
Ssc.16800.1.A1_at	BFS	-0.393951535	4.19215E-05
Ssc.16823.1.S1_at	BW150	-0.331139114	0.000814182
Ssc.16895.1.S1_at	BF75	-0.331247805	0.000672014
Ssc.16988.1.S1_at	HW	-0.347401343	0.000426534
Ssc.16991.1.A1_at	HW	0.348815659	0.000402543
Ssc.17193.1.A1_at	BFS	-0.386903979	5.89465E-05
Ssc.17228.1.S1_at	LBW	-0.360442844	0.000247527
Ssc.17306.1.A1_at	BF75	-0.33523467	0.000572741

Ssc.17314.1.S1_at	HW	0.326435318	0.000975279
Ssc.17315.1.S1_at	HW	0.349330047	0.000394129
Ssc.17458.1.S1_at	BW150	-0.339755617	0.000580564
Ssc.17510.1.S1_at	BF75	-0.466978944	7.51338E-07
Ssc.17559.1.A1_at	BW150	-0.457419497	1.93743E-06
Ssc.17602.1.S1_at	BW150	0.363833468	0.000214046
Ssc.17629.1.A1_at	HW	-0.367101441	0.000185784
Ssc.1775.2.S1_at	HW	0.399370403	4.22222E-05
Ssc.17785.1.S1_at	BF75	-0.324636931	0.000871877
Ssc.17788.1.A1_at	BF75	-0.446972298	2.48466E-06
Ssc.17793.1.S1_at	BW150	-0.354203705	0.000322069
Ssc.17794.1.A1_at	BF75	-0.415647731	1.39693E-05
Ssc.17832.1.S1_at	BF75	-0.34990774	0.000312149
Ssc.17840.2.A1_at	BF75	-0.411218151	1.75922E-05
Ssc.17844.1.A1_at	BF75	-0.350778125	0.000300829
Ssc.17850.1.A1_at	BF75	-0.443459078	3.0418E-06
Ssc.17853.1.A1_at	IMF	-0.343929036	0.000429133
Ssc.1790.1.S1_at	HW	-0.356118355	0.000297244
Ssc.1790.3.A1_at	BF75	-0.35175914	0.000288524
Ssc.17953.1.S1_at	HW	-0.33980808	0.000579353
Ssc.17991.1.A1_at	BW150	-0.365840352	0.000196254
Ssc.18076.1.A1_at	BFS	-0.392516309	4.49628E-05
Ssc.18078.1.A1_at	HW	0.356935757	0.000287193
Ssc.18121.1.A1_at	BFS	-0.339990542	0.000471984
Ssc.18126.1.S1_at	BFS	-0.328929507	0.000736749
Ssc.18164.1.A1_at	BW150	-0.363713562	0.000215154
Ssc.18200.1.S1_at	BF75	-0.378415504	8.79701E-05
Ssc.18212.1.S1_at	BF75	-0.364968809	0.000162243
Ssc.18300.1.A1_at	HW	0.355481263	0.000305302
Ssc.18302.2.S1_at	HW	0.344870065	0.00047278
Ssc.1833.1.A1_at	LBW	0.341176708	0.000548554
Ssc.18335.1.S1_at	HW	-0.378063039	0.000114261
Ssc.18338.1.A1_at	HW	0.330115687	0.000847006
Ssc.18369.1.A1_at	BF75	-0.402277337	2.77457E-05
Ssc.18395.1.A1_at	BF75	-0.35116111	0.000295969
Ssc.18424.1.A1_at	BFS	-0.352609373	0.000278237
Ssc.1844.1.S1_at	BF75	0.33624031	0.000549922
Ssc.18443.2.A1_at	BF75	-0.40466452	2.45989E-05
Ssc.18466.2.S1_at	BF75	-0.416771827	1.31685E-05
Ssc.1848.1.A1_at	LBW	-0.328531972	0.000900187
Ssc.18480.1.S1_at	BF75	0.35275966	0.000276454
Ssc.18488.1.S1_at	BFS	-0.371387432	0.000121542
Ssc.18537.2.S1_at	BF75	-0.380429286	8.00785E-05
Ssc.18552.1.A1_x_at	BW150	-0.464025957	1.3144E-06
Ssc.18561.2.A1_at	BF75	-0.340160813	0.000468699
Ssc.18571.1.S1_at	BFS	-0.334059137	0.000600515
Ssc.1860.1.S1_at	HW	-0.374940802	0.00013146
Ssc.18619.2.A1_at	BF75	0.366007558	0.000154897
Ssc.18628.1.A1_at	BF75	-0.363015687	0.000176937
Ssc.18640.2.S1_at	HW	0.330715165	0.000827636
Ssc.18678.1.A1_at	BF75	-0.387809678	5.64447E-05
Ssc.18753.1.A1_at	BFS	-0.34655685	0.000359499
Ssc.18767.1.A1_at	BFS	-0.376619611	9.56124E-05
Ssc.18769.1.A1_at	BFS	-0.363492355	0.000173241
Ssc.18772.1.A1_at	BFS	-0.327920243	0.000766673
Ssc.18794.1.A1_at	BFS	-0.356955899	0.000230737
Ssc.18795.1.A1_at	BF75	-0.428414092	7.05543E-06
Ssc.18817.1.A1_at	BFS	-0.38035552	8.03555E-05
Ssc.18827.1.A1_at	BF75	-0.374132993	0.000107217
Ssc.18838.1.A1_at	HW	-0.327414037	0.000939543
Ssc.18856.1.A1_at	HW	-0.350869631	0.000369904
Ssc.1888.2.S1_at	LBW	0.330236436	0.000843071
Ssc.18939.1.A1_at	BFS	-0.322979929	0.000929831
Ssc.18966.1.A1_at	BFS	-0.431918723	5.82049E-06
Ssc.190.1.S1_at	BF75	-0.450037512	2.07876E-06
Ssc.19001.1.A1_at	BFS	-0.328669267	0.000744361

Ssc.19025.1.A1_at	BFS	-0.325927159	0.000829056
Ssc.19026.1.A1_at	BF75	-0.471170488	5.79128E-07
Ssc.19045.1.S1_at	IMF	-0.378497985	9.51077E-05
Ssc.19049.1.A1_at	BFS	-0.349374347	0.00031928
Ssc.19119.1.A1_at	BFS	-0.339917341	0.000473403
Ssc.19122.1.A1_at	BFS	-0.374080754	0.000107474
Ssc.19141.1.S1_at	IMF	-0.443677868	3.36756E-06
Ssc.19181.2.A1_at	BFS	-0.327915036	0.000766831
Ssc.19193.2.S1_at	BF75	0.356054169	0.000239927
Ssc.19197.1.S1_at	BW150	-0.445237116	3.88021E-06
Ssc.1920.1.A1_at	HW	-0.329348659	0.000872394
Ssc.19208.2.S1_at	BFS	-0.321625821	0.000979778
Ssc.19211.1.S1_at	BF75	-0.401505679	2.8841E-05
Ssc.19237.1.S1_at	BF75	-0.322314768	0.000954068
Ssc.1924.1.S1_at	BF75	0.32249632	0.000947396
Ssc.19284.2.S1_at	BF75	-0.323099148	0.000925547
Ssc.19288.2.S1_at	IMF	-0.34109629	0.000481933
Ssc.19394.1.A1_at	BF75	-0.401923925	2.82424E-05
Ssc.19397.1.A1_at	BF75	-0.33567197	0.000562714
Ssc.19422.1.S1_at	BFS	-0.324900139	0.000862981
Ssc.19470.1.A1_at	BF75	-0.525094509	1.46305E-08
Ssc.19498.1.A1_at	BFS	-0.366787635	0.000149584
Ssc.19499.1.A1_at	BF75	-0.461312593	1.06245E-06
Ssc.19514.2.A1_at	BF75	-0.395051789	3.97213E-05
Ssc.19524.1.S1_at	BW150	-0.49396676	2.03833E-07
Ssc.19548.2.A1_at	BF75	-0.560827069	8.76405E-10
Ssc.19558.1.S1_at	HW	0.334227386	0.000722048
Ssc.19574.2.S1_at	BFS	-0.345801648	0.000371045
Ssc.19586.1.S1_at	BF75	-0.514096212	3.26474E-08
Ssc.19590.1.S1_at	BW150	-0.502732088	1.14118E-07
Ssc.19609.1.S1_at	BW150	0.395685736	5.04009E-05
Ssc.19610.1.S1_at	BF75	0.334163583	0.000597998
Ssc.19610.2.A1_at	HW	0.341783507	0.000535386
Ssc.1962.1.S1_at	HW	-0.335686856	0.000681915
Ssc.19634.1.S1_at	BW150	0.348654578	0.000405211
Ssc.19689.1.S1_at	BW150	-0.376444825	0.000122896
Ssc.19695.1.S1_at	BF75	-0.344512446	0.000391548
Ssc.19702.1.A1_at	BFS	-0.336492257	0.000544337
Ssc.19736.1.S1_at	BF75	-0.410723895	1.80471E-05
Ssc.19779.1.S1_at	LBW	0.329975918	0.000851581
Ssc.19817.1.S1_at	BFS	-0.344480311	0.000392072
Ssc.19842.1.S1_at	BW150	-0.416069375	1.84278E-05
Ssc.19876.1.A1_at	BF75	-0.50035833	8.55685E-08
Ssc.2015.1.A1_at	BF75	-0.372161677	0.000117332
Ssc.20162.1.S1_at	HW	0.371111526	0.000155829
Ssc.20217.2.S1_at	HW	0.33629806	0.000665723
Ssc.20317.1.S1_at	BF75	-0.529983697	1.01456E-08
Ssc.20392.1.S1_at	HW	-0.419882207	1.51544E-05
Ssc.20445.1.S1_at	HW	-0.41937464	1.55563E-05
Ssc.20525.1.S1_at	HW	0.341479644	0.000541944
Ssc.2062.1.A1_at	HW	0.362994401	0.000221917
Ssc.20639.1.S1_at	BF75	-0.393642499	4.25596E-05
Ssc.20653.1.S1_at	BF75	-0.331752389	0.000658634
Ssc.20685.1.S1_at	HW	-0.343201898	0.000505728
Ssc.20755.1.S1_at	IMF	-0.341862238	0.000467097
Ssc.20827.1.S1_at	HW	-0.334080743	0.000726198
Ssc.2091.1.S1_at	BW150	-0.382954611	9.14628E-05
Ssc.20922.1.S1_at	BF75	-0.337950397	0.000513025
Ssc.20966.1.S1_at	IMF	0.323509149	0.000966955
Ssc.21050.1.A1_at	IMF	-0.324235145	0.000940315
Ssc.21074.1.A1_at	BF75	0.358669193	0.000214166
Ssc.21139.1.S1_at	HW	0.459554388	1.71065E-06
Ssc.21139.2.S1_at	HW	0.35315589	0.00033645
Ssc.21145.1.S1_at	IMF	-0.358750686	0.000229653
Ssc.2116.1.A1_at	BF75	-0.329177365	0.000729565
Ssc.21181.2.S1_at	BFS	-0.323641641	0.000906278

Ssc.21215.1.S1_at	BF75	-0.327694053	0.000773531
Ssc.21231.2.S1_at	IMF	-0.358178026	0.000235403
Ssc.21242.1.S1_at	LBW	-0.350528708	0.000375147
Ssc.21253.1.S1_at	BFS	-0.35894862	0.00021157
Ssc.2131.2.A1_at	BW150	-0.342236813	0.000525738
Ssc.21361.1.A1_at	BW150	-0.329876336	0.000854855
Ssc.21404.1.A1_at	BFS	-0.364233517	0.000167637
Ssc.21450.1.S1_at	LBW	0.340763643	0.000557688
Ssc.2152.1.A1_at	HW	-0.326829955	0.000960723
Ssc.21529.1.A1_at	BF75	-0.51282767	3.57492E-08
Ssc.21543.1.S1_at	HW	-0.327095683	0.000951034
Ssc.21565.2.S1_at	BF75	-0.555511238	1.36077E-09
Ssc.2157.1.A1_at	BFS	-0.344402178	0.000393349
Ssc.21584.1.S1_at	BF75	-0.393452799	4.29558E-05
Ssc.21608.1.S1_at	HW	0.341586497	0.000539629
Ssc.2167.2.S1_at	BW150	-0.547770878	4.41363E-09
Ssc.21696.1.S1_at	IMF	-0.336179778	0.000587938
Ssc.21699.1.A1_at	BFS	-0.378871732	8.61214E-05
Ssc.21713.1.A1_at	BF75	0.36829071	0.000139821
Ssc.21746.1.S1_at	BF75	-0.331030952	0.000677841
Ssc.21798.2.S1_a_at	BF75	-0.434096992	5.15882E-06
Ssc.21826.1.S1_at	BF75	-0.332276542	0.000644993
Ssc.21829.2.S1_at	HW	0.341255879	0.00054682
Ssc.21858.2.A1_at	BF75	-0.487495591	2.03147E-07
Ssc.21861.1.S1_at	HW	0.332687515	0.000766724
Ssc.21903.1.S1_at	BF75	-0.333450032	0.000615386
Ssc.21911.1.S1_at	BF75	-0.431590028	5.92702E-06
Ssc.21930.1.S1_a_at	BF75	0.369266659	0.000133802
Ssc.21969.1.A1_at	HW	0.4091343	2.6142E-05
Ssc.21976.1.S1_at	BF75	0.363122402	0.000176103
Ssc.22041.1.S1_at	BF75	-0.454471384	1.60109E-06
Ssc.22077.1.S1_at	HW	0.45976029	1.69016E-06
Ssc.22088.1.S1_at	HW	-0.39809634	4.48986E-05
Ssc.22089.1.A1_at	HW	-0.333860161	0.000732481
Ssc.22118.1.A1_at	BF75	-0.47869942	3.59649E-07
Ssc.22143.2.S1_at	BF75	-0.46445658	8.77307E-07
Ssc.22152.1.A1_at	HW	0.336141131	0.000669847
Ssc.22157.1.A1_at	BF75	-0.346096586	0.000366496
Ssc.22165.1.A1_at	BFS	-0.328183081	0.000758775
Ssc.2217.1.S1_at	HW	0.341894968	0.000532999
Ssc.2217.2.S1_at	HW	0.382485548	9.34499E-05
Ssc.22183.1.A1_at	HW	-0.330632907	0.000830269
Ssc.22220.2.S1_at	HW	0.342182598	0.000526884
Ssc.22229.1.A1_at	HW	-0.333658494	0.000738269
Ssc.22262.1.A1_at	BF75	-0.526336469	1.33387E-08
Ssc.22347.1.A1_at	BF75	-0.383327029	6.98731E-05
Ssc.22371.1.A1_at	BF75	-0.342757916	0.000421128
Ssc.22400.1.A1_at	HW	-0.34190586	0.000532766
Ssc.22445.1.A1_at	BFS	-0.354741375	0.000253912
Ssc.22466.1.A1_at	BF75	-0.519820654	2.15753E-08
Ssc.22499.1.A1_at	HW	-0.359468413	0.000258007
Ssc.22523.1.A1_at	BF75	-0.392854154	4.42289E-05
Ssc.22529.1.A1_at	BFS	-0.355438227	0.000246397
Ssc.22538.1.A1_at	BF75	-0.363334142	0.00017446
Ssc.22617.1.A1_at	BF75	-0.390544252	4.94789E-05
Ssc.22621.1.S1_at	IMF	-0.36805266	0.000152684
Ssc.22641.1.S1_at	BF75	0.349820391	0.000313307
Ssc.22685.1.S1_at	BF75	-0.322558833	0.000945108
Ssc.22701.1.S1_at	BFS	-0.357500841	0.000225342
Ssc.22766.1.A1_at	BFS	-0.383113387	7.0582E-05
Ssc.22770.1.A1_at	BF75	0.354820476	0.000253048
Ssc.22789.1.S1_at	BW150	-0.554151128	2.67566E-09
Ssc.23124.1.S1_at	BW150	-0.333195568	0.000751713
Ssc.23128.1.S1_at	BW150	0.339265189	0.000592003
Ssc.23148.1.S1_at	BF75	-0.321737812	0.000975556
Ssc.2318.1.A1_at	BF75	-0.323091563	0.000925819

Ssc.23205.1.A1_at	BF75	-0.473957507	4.86142E-07
Ssc.23266.1.A1_at	BFS	-0.342878723	0.000419027
Ssc.23360.1.A1_at	BF75	-0.395672553	3.85281E-05
Ssc.23364.1.A1_at	BW150	-0.375323822	0.000129228
Ssc.23472.1.S1_at	HW	0.365388279	0.000200138
Ssc.23484.1.A1_a_at	HW	-0.332089218	0.000784754
Ssc.23501.1.S1_s_at	BW150	0.362058716	0.00023101
Ssc.23521.2.S1_at	BFS	-0.397580409	3.50672E-05
Ssc.2353.1.S1_at	BF75	0.364075365	0.000168818
Ssc.23540.1.A1_at	BF75	-0.351133901	0.000296311
Ssc.2357.1.S1_at	HW	-0.326730552	0.000964371
Ssc.23632.1.S1_at	LBW	-0.326201256	0.000984007
Ssc.23761.2.S1_at	HW	0.330187864	0.000844652
Ssc.23898.1.A1_at	BW150	-0.384155199	8.65542E-05
Ssc.2393.1.S1_at	IMF	-0.433648462	5.89693E-06
Ssc.23936.2.S1_a_at	BW150	-0.46271224	1.42074E-06
Ssc.24006.2.A1_at	HW	-0.360702721	0.000244799
Ssc.24023.1.A1_at	BFS	-0.323231983	0.000920794
Ssc.24185.1.A1_at	HW	-0.330003769	0.000850668
Ssc.24216.2.S1_at	BF75	-0.429387218	6.68981E-06
Ssc.24253.1.S1_at	BF75	-0.372417192	0.000115972
Ssc.24328.1.S1_at	BFS	0.370435834	0.000126908
Ssc.24383.1.S1_at	HW	-0.326987799	0.000954957
Ssc.24403.1.S1_at	IMF	0.399925253	3.42101E-05
Ssc.2441.1.S1_at	BW150	-0.394130096	5.42791E-05
Ssc.2441.2.A1_at	HW	-0.345806589	0.000455159
Ssc.24438.1.A1_at	BFS	-0.346945234	0.00035369
Ssc.2444.2.A1_a_at	BFS	-0.333636091	0.000610808
Ssc.24490.2.A1_at	HW	-0.339972419	0.000575573
Ssc.24522.1.A1_at	BF75	-0.391886213	4.63621E-05
Ssc.24539.1.A1_at	LBW	0.347426257	0.0004261
Ssc.24542.1.S1_at	HW	0.332092939	0.00078464
Ssc.24608.1.S1_at	HW	0.332149542	0.000782919
Ssc.24613.1.A1_at	BFS	-0.363242963	0.000175166
Ssc.24647.1.A1_at	BF75	-0.351942683	0.000286274
Ssc.24656.1.A1_at	LBW	-0.347020931	0.000433208
Ssc.24708.1.S1_at	BF75	-0.524893268	1.48508E-08
Ssc.2472.3.S1_at	BF75	-0.407335523	2.14756E-05
Ssc.24724.1.A1_at	HW	-0.43798812	5.7929E-06
Ssc.24748.1.A1_at	BF75	-0.389878379	5.1097E-05
Ssc.24753.1.S1_at	HW	-0.419580175	1.53924E-05
Ssc.24769.1.A1_at	BW150	-0.338356202	0.000613751
Ssc.24814.1.S1_at	HW	0.366183695	0.000193351
Ssc.24997.1.S1_at	BF75	-0.375300736	0.000101613
Ssc.25019.1.A1_a_at	IMF	0.331200572	0.000716708
Ssc.25025.1.S1_at	BF75	-0.345506089	0.000375656
Ssc.25076.2.S1_at	BF75	-0.488507029	1.90037E-07
Ssc.25089.1.S1_at	BF75	-0.343347723	0.000410962
Ssc.25094.1.A1_at	BF75	-0.445280101	2.73975E-06
Ssc.25100.1.S1_at	HW	-0.375930343	0.000125765
Ssc.25117.1.A1_at	BF75	-0.357856678	0.000221882
Ssc.25143.1.A1_at	BF75	-0.399915078	3.12276E-05
Ssc.2515.1.S1_at	HW	0.337683967	0.000630302
Ssc.2516.2.S1_a_at	HW	0.356007964	0.000298626
Ssc.25219.2.S1_at	BFS	-0.367244841	0.00014655
Ssc.25264.2.S1_a_at	BFS	-0.389949208	5.09225E-05
Ssc.25314.2.S1_at	BF75	-0.465823142	8.06766E-07
Ssc.25350.1.A1_at	BFS	-0.339334329	0.000484843
Ssc.25351.1.S1_at	BW150	-0.462125456	1.47083E-06
Ssc.2537.1.A1_at	BF75	-0.356419774	0.000236161
Ssc.25378.1.S1_at	BW150	-0.431750225	8.11891E-06
Ssc.25387.1.S1_at	HW	-0.330330715	0.000840011
Ssc.25391.1.S1_at	BW150	-0.370869135	0.000157504
Ssc.25423.1.S1_at	HW	0.326899944	0.000958162
Ssc.25498.1.S1_at	IMF	0.340643399	0.000490907
Ssc.25555.1.S1_at	BW150	-0.35497452	0.000311854

Ssc.25573.1.S1_at	HW	-0.338433772	0.000611867
Ssc.25656.1.A1_at	BF75	-0.333975083	0.000602547
Ssc.25740.1.A1_at	HW	-0.371754555	0.000151464
Ssc.25751.1.S1_at	BF75	-0.369453246	0.000132679
Ssc.25766.1.S1_at	HW	-0.340922992	0.000554148
Ssc.25798.1.S1_at	BF75	-0.35783783	0.000222064
Ssc.25928.2.S1_at	BFS	-0.377360107	9.23894E-05
Ssc.26011.1.S1_at	BF75	-0.373274349	0.000111518
Ssc.26033.1.S1_at	BF75	-0.332304982	0.00064426
Ssc.26101.1.S1_at	BF75	-0.347664462	0.000343162
Ssc.26118.1.S1_at	BF75	-0.502522377	7.37244E-08
Ssc.26309.1.A1_at	BW150	-0.335910795	0.000675941
Ssc.26316.1.S1_at	HW	-0.388807782	6.97544E-05
Ssc.26374.1.S1_at	BFS	-0.33658552	0.000542283
Ssc.26403.1.S1_at	BFS	-0.343507047	0.000408255
Ssc.26431.1.A1_at	BW150	-0.417443696	1.71782E-05
Ssc.26449.3.S1_a_at	BF75	-0.354577215	0.000255713
Ssc.26458.1.A1_at	BF75	-0.363867312	0.000170385
Ssc.26493.1.A1_at	BF75	-0.328440897	0.0007511
Ssc.26517.2.A1_at	BF75	-0.369568929	0.000131987
Ssc.26530.1.S1_at	HW	0.363557052	0.00021661
Ssc.26538.1.A1_at	BF75	-0.414816556	1.45907E-05
Ssc.26603.1.S1_at	IMF	-0.393090384	4.7768E-05
Ssc.26610.1.A1_at	BFS	-0.350907566	0.000299178
Ssc.26734.1.A1_at	BFS	-0.324950204	0.000861298
Ssc.26788.1.S1_at	HW	-0.346051013	0.00045066
Ssc.26791.1.S1_at	BFS	-0.323603657	0.000907616
Ssc.26854.1.S1_at	BFS	-0.326013267	0.000826268
Ssc.26895.1.A1_at	BW150	-0.399581999	4.17924E-05
Ssc.26900.1.A1_at	LBW	-0.337558342	0.00063344
Ssc.26903.1.A1_at	BF75	-0.333424273	0.000616023
Ssc.26904.1.A1_at	BF75	-0.383098514	7.06316E-05
Ssc.26940.1.A1_at	BFS	-0.342618992	0.000423555
Ssc.26972.1.A1_at	BFS	-0.327250954	0.000787126
Ssc.2701.2.S1_at	BF75	0.36231376	0.000182512
Ssc.27042.1.A1_at	BFS	-0.330239649	0.000699497
Ssc.27046.1.A1_at	BFS	-0.369742001	0.000130958
Ssc.27076.1.A1_at	BF75	-0.460688143	1.10338E-06
Ssc.27118.1.A1_at	BF75	-0.61568453	5.71476E-12
Ssc.27125.1.A1_at	BF75	-0.347060498	0.000351983
Ssc.27150.1.S1_at	BF75	-0.342893883	0.000418764
Ssc.27185.1.S1_at	BFS	-0.458426702	1.26448E-06
Ssc.27198.1.A1_at	BW150	-0.335783847	0.000679322
Ssc.27209.1.S1_at	BW150	0.344370408	0.000482436
Ssc.27233.1.S1_at	HW	0.332746445	0.000764969
Ssc.27301.1.S1_at	BF75	-0.416928017	1.30607E-05
Ssc.27320.1.S1_at	HW	0.330615164	0.000830838
Ssc.27325.1.S1_at	HW	-0.376550968	0.000122311
Ssc.27358.2.A1_at	BFS	-0.4184924	1.20258E-05
Ssc.2736.2.S1_at	HW	0.350667782	0.000373
Ssc.27383.1.A1_at	IMF	-0.41197041	1.86553E-05
Ssc.27387.1.A1_at	BFS	-0.325252961	0.000851186
Ssc.27400.1.A1_at	BF75	-0.368391859	0.000139186
Ssc.27427.1.S1_a_at	BW150	-0.492996034	2.17142E-07
Ssc.27429.1.A1_at	HW	-0.342839239	0.000513164
Ssc.27476.1.S1_at	BW150	-0.575660187	4.56955E-10
Ssc.27524.1.S1_at	BF75	-0.359861573	0.00020329
Ssc.27540.2.S1_at	BW150	-0.513945645	5.30402E-08
Ssc.27578.1.S1_at	BFS	-0.353126442	0.000272147
Ssc.27586.1.S1_at	BF75	-0.472912857	5.19198E-07
Ssc.27586.2.S1_at	BW150	-0.39212029	5.9702E-05
Ssc.27633.1.S1_at	HW	-0.335689669	0.00068184
Ssc.27637.1.S1_at	IMF	-0.349881112	0.00033508
Ssc.2792.1.S1_at	HW	0.327751203	0.000927511
Ssc.27938.1.S1_at	HW	0.34418419	0.000486081
Ssc.27952.1.A1_at	BW150	-0.496840704	1.6883E-07

Ssc.27979.1.A1_at	BF75	-0.34372155	0.000404637
Ssc.28033.1.A1_at	BW150	-0.347806736	0.000419526
Ssc.28034.1.A1_at	BFS	-0.371024709	0.000123562
Ssc.28045.1.A1_at	BFS	-0.35389173	0.00026336
Ssc.28087.1.A1_at	BF75	-0.413108573	1.59498E-05
Ssc.28091.2.A1_a_at	BFS	-0.377120414	9.34215E-05
Ssc.28122.1.A1_at	BFS	-0.355237833	0.000248537
Ssc.28150.1.S1_at	BF75	0.406755676	2.21202E-05
Ssc.28152.1.A1_at	BFS	-0.399623289	3.16849E-05
Ssc.28171.1.A1_at	BFS	-0.381835914	7.49627E-05
Ssc.28203.1.A1_at	BF75	-0.438145171	4.11324E-06
Ssc.28236.1.A1_a_at	BFS	-0.325580037	0.000840384
Ssc.28263.1.A1_at	BF75	-0.490570191	1.65748E-07
Ssc.28265.1.A1_at	BFS	-0.362976123	0.000177247
Ssc.28282.1.A1_at	BFS	-0.328625668	0.000745643
Ssc.2830.1.A1_at	BFS	-0.358399562	0.000216699
Ssc.28302.1.A1_at	BF75	-0.32175273	0.000974995
Ssc.28330.1.S1_at	BFS	-0.328116877	0.000760758
Ssc.28350.1.A1_at	BF75	-0.475176905	4.50084E-07
Ssc.28379.1.A1_at	BFS	-0.331582083	0.000663122
Ssc.28414.1.A1_at	BF75	-0.322088445	0.000962445
Ssc.28451.1.A1_at	BW150	-0.398949692	4.3089E-05
Ssc.28457.1.S1_at	BF75	0.349839126	0.000313058
Ssc.28483.2.A1_at	BF75	-0.359865454	0.000203256
Ssc.28502.1.S1_at	HW	-0.471382417	8.451E-07
Ssc.28512.1.S1_at	BW150	-0.366445695	0.000191162
Ssc.28602.1.A1_at	BFS	-0.375737099	9.959E-05
Ssc.28670.1.S1_at	BF75	-0.455202999	1.53304E-06
Ssc.28690.2.S1_at	HW	0.363013443	0.000221735
Ssc.28690.3.S1_at	HW	0.452696003	2.54424E-06
Ssc.28692.1.S1_at	BW150	-0.327830764	0.000924693
Ssc.2871.1.S1_at	IMF	0.336610009	0.000577872
Ssc.28782.3.S1_at	BF75	-0.419169172	1.16024E-05
Ssc.28893.1.A1_at	HW	0.379989294	0.000104717
Ssc.28961.1.S1_at	IMF	-0.324512867	0.000930303
Ssc.29017.1.A1_at	BW150	-0.331631739	0.0007988
Ssc.29026.1.S1_at	BF75	-0.360940918	0.000193889
Ssc.29058.2.A1_at	LBW	-0.34051738	0.000563199
Ssc.29106.1.S1_at	IMF	-0.33374613	0.000647962
Ssc.29164.1.A1_at	BFS	0.32776466	0.000771384
Ssc.29169.1.A1_at	HW	-0.347332425	0.000427736
Ssc.29174.1.A1_at	BW150	-0.445438204	3.8368E-06
Ssc.29204.1.A1_at	BF75	-0.350910663	0.000299139
Ssc.29209.1.A1_at	BF75	-0.4057674	2.32609E-05
Ssc.29216.1.A1_at	BFS	-0.327541019	0.000778202
Ssc.2923.1.S1_at	HW	0.36038644	0.000248123
Ssc.2925.3.S1_a_at	BW150	0.332159985	0.000782601
Ssc.29250.1.A1_at	BFS	-0.326926666	0.000797215
Ssc.29315.1.A1_at	BF75	-0.350449307	0.00030506
Ssc.29319.1.A1_at	BFS	-0.365921347	0.000155495
Ssc.29358.1.A1_at	BFS	-0.357761816	0.0002228
Ssc.29388.1.A1_at	LBW	-0.334512129	0.000714053
Ssc.29413.1.A1_at	BFS	-0.332270889	0.000645138
Ssc.29467.1.A1_at	IMF	-0.348994807	0.000347762
Ssc.29478.1.A1_at	BF75	-0.452833836	1.76392E-06
Ssc.2949.1.S1_at	HW	0.329558875	0.000865368
Ssc.29503.1.A1_at	BFS	-0.378131117	8.91412E-05
Ssc.29517.1.A1_at	BFS	-0.391975017	4.61625E-05
Ssc.29521.1.A1_at	BF75	-0.452417301	1.80777E-06
Ssc.29532.1.A1_at	BFS	-0.343427321	0.000409608
Ssc.29539.1.A1_at	BFS	-0.322635206	0.000942321
Ssc.29592.1.A1_at	BFS	-0.343846133	0.000402548
Ssc.29595.1.A1_at	BFS	-0.347814316	0.000341005
Ssc.29634.1.A1_at	HW	-0.337312239	0.00063963
Ssc.29635.1.A1_at	BF75	-0.340986061	0.000453073
Ssc.29654.1.A1_at	BW150	-0.451394638	2.74063E-06

Ssc.29676.2.A1_a_at	BW150	-0.34966435	0.000388748
Ssc.2968.1.S1_at	HW	0.428081123	9.87153E-06
Ssc.29722.1.S1_at	BFS	-0.32907809	0.000732435
Ssc.29726.1.A1_at	BF75	-0.406791453	2.20799E-05
Ssc.29776.1.A1_at	BF75	-0.331333352	0.000669728
Ssc.29793.1.A1_at	BF75	-0.351808281	0.00028792
Ssc.298.1.S1_at	BF75	-0.340791635	0.000456711
Ssc.29846.1.A1_at	HW	-0.339172339	0.000594191
Ssc.29860.1.A1_at	BFS	-0.341042904	0.000452015
Ssc.29864.1.A1_at	IMF	0.354657324	0.000273773
Ssc.29872.1.A1_at	HW	-0.37598928	0.000125433
Ssc.29880.1.A1_s_at	HW	-0.327889963	0.0009226
Ssc.29932.1.A1_at	BFS	-0.339997266	0.000471854
Ssc.29937.1.A1_at	BFS	-0.344609679	0.000389966
Ssc.29951.2.A1_at	BF75	-0.462035431	1.01686E-06
Ssc.29952.2.A1_at	IMF	0.325603742	0.000891908
Ssc.30017.1.A1_at	BFS	-0.413595222	1.55509E-05
Ssc.30074.1.A1_at	IMF	0.327089205	0.00084195
Ssc.30078.1.A1_at	BF75	-0.344942273	0.000384599
Ssc.30127.1.A1_at	BF75	-0.373209973	0.000111847
Ssc.30140.1.A1_at	BFS	-0.407155321	2.1674E-05
Ssc.30164.1.A1_at	BFS	-0.341670163	0.000440484
Ssc.30179.1.A1_at	HW	-0.404683852	3.2587E-05
Ssc.30182.1.A1_at	BF75	0.342253513	0.000430004
Ssc.30231.1.A1_at	BFS	-0.3298613	0.000710074
Ssc.30237.1.A1_at	BFS	-0.379542032	8.34706E-05
Ssc.30255.1.A1_at	BFS	-0.345851883	0.000370267
Ssc.30278.1.A1_at	BF75	-0.383813225	6.82844E-05
Ssc.3028.1.S1_at	IMF	-0.324589683	0.00092755
Ssc.30322.1.A1_at	BFS	-0.361833157	0.000186422
Ssc.30377.1.A1_at	HW	-0.343443621	0.000500827
Ssc.30467.1.A1_at	BF75	-0.337506354	0.00052238
Ssc.30516.1.A1_at	BF75	-0.539822865	4.77072E-09
Ssc.30517.1.A1_at	BF75	-0.373977116	0.000107986
Ssc.30528.1.A1_at	BF75	-0.323110522	0.000925139
Ssc.3055.2.S1_at	BF75	-0.37764423	9.11798E-05
Ssc.30593.1.A1_at	BFS	-0.348169763	0.000335938
Ssc.30633.1.S1_at	LBW	-0.335577337	0.000684855
Ssc.30651.1.A1_at	BFS	-0.347067769	0.000351876
Ssc.30715.1.S1_at	HW	0.336175655	0.000668937
Ssc.30777.1.S1_a_at	BW150	-0.330607976	0.000831069
Ssc.30813.1.A1_at	BFS	0.475443457	4.42548E-07
Ssc.30830.2.A1_at	BF75	-0.353579264	0.000266916
Ssc.30911.1.S1_at	BF75	-0.508522789	4.85114E-08
Ssc.3092.1.S1_at	IMF	-0.326614804	0.00085762
Ssc.30924.1.S1_at	HW	-0.337666794	0.000630731
Ssc.30965.1.A1_at	BFS	-0.351801952	0.000287998
Ssc.30987.1.S1_at	HW	-0.348242254	0.000412116
Ssc.3106.1.S1_at	IMF	-0.335957551	0.000593199
Ssc.31076.1.A1_at	LBW	0.34400485	0.000489615
Ssc.31079.1.A1_at	BF75	-0.333828567	0.000606105
Ssc.31119.1.A1_at	BF75	-0.362600318	0.000180217
Ssc.31137.1.A1_at	BW150	-0.347713768	0.000421124
Ssc.31187.1.S1_at	HW	0.370263698	0.000161761
Ssc.31200.1.S1_at	BF75	-0.412453452	1.65019E-05
Ssc.3217.1.S1_at	BF75	-0.358655705	0.000214292
Ssc.3219.1.S1_at	HW	0.343307124	0.000503589
Ssc.3227.1.A1_at	BW150	-0.342698065	0.000516086
Ssc.323.1.S1_at	BF75	-0.447882258	2.35695E-06
Ssc.3249.1.S1_at	BW150	0.360572871	0.000246158
Ssc.3481.2.S1_at	HW	-0.379540779	0.000106871
Ssc.3538.1.S1_at	HW	-0.340233055	0.000569624
Ssc.3564.1.A1_at	BFS	-0.411598037	1.72499E-05
Ssc.3574.1.A1_at	HW	-0.347071051	0.000432323
Ssc.3577.1.S1_at	BF75	-0.4191266	1.16286E-05
Ssc.3582.1.S1_at	HW	-0.340958231	0.000553368

Ssc.3584.1.S1_at	BFS	-0.357219919	0.000228108
Ssc.3596.1.S1_at	BF75	-0.324723678	0.000868936
Ssc.3717.1.S1_at	HW	0.371061035	0.000156176
Ssc.3799.3.A1_at	IMF	-0.333620765	0.000651201
Ssc.384.1.A1_a_at	BF75	-0.454420824	1.6059E-06
Ssc.3884.1.S1_at	IMF	-0.37103085	0.000133625
Ssc.3930.1.S1_at	BF75	-0.481435026	3.01628E-07
Ssc.3931.1.S1_at	HW	0.333843483	0.000732958
Ssc.3966.1.S1_at	BFS	-0.33546102	0.000567531
Ssc.3981.1.A1_at	HW	0.340870559	0.00055531
Ssc.3999.1.S1_at	BF75	-0.437534854	4.25692E-06
Ssc.4035.1.A1_at	BFS	-0.386753834	5.93711E-05
Ssc.4143.2.S1_at	HW	0.346619364	0.000440357
Ssc.4194.1.S1_at	BF75	-0.323794318	0.000900922
Ssc.420.1.S1_a_at	BF75	-0.481291081	3.04445E-07
Ssc.4223.2.S1_at	HW	0.344030383	0.00048911
Ssc.4246.2.S1_at	HW	0.335285392	0.000692746
Ssc.4251.1.S1_at	BF75	-0.347302619	0.000348422
Ssc.428.16.A1_at	BF75	-0.345957271	0.000368638
Ssc.4288.1.S1_a_at	HW	-0.418787926	1.60332E-05
Ssc.4329.1.S1_at	BFS	-0.348583638	0.000330126
Ssc.437.1.S1_a_at	BFS	-0.32802931	0.000763387
Ssc.4378.1.A1_at	IMF	-0.34256602	0.000453836
Ssc.4441.3.A1_at	BF75	-0.567302627	5.07385E-10
Ssc.4475.2.A1_at	BFS	-0.351378666	0.00029324
Ssc.4497.2.A1_at	BFS	-0.405349922	2.37591E-05
Ssc.4515.1.S1_at	HW	0.378981599	0.000109613
Ssc.4519.1.A1_at	BF75	-0.330925456	0.000680692
Ssc.4669.1.S1_at	BF75	0.328780912	0.000741087
Ssc.4725.1.A1_at	HW	0.340222022	0.000569875
Ssc.4820.1.S1_at	IMF	-0.334847559	0.000620133
Ssc.4852.2.S1_a_at	IMF	0.349615939	0.000338829
Ssc.4881.1.S1_at	BW150	-0.454354692	2.31317E-06
Ssc.4924.1.S1_at	LBW	0.342528724	0.000519611
Ssc.4925.1.S1_at	BF75	0.330382245	0.000695548
Ssc.4927.1.A1_at	IMF	0.34991359	0.000334623
Ssc.4956.1.A1_at	BW150	-0.40084682	3.93073E-05
Ssc.4960.1.S1_at	HW	0.380869479	0.000100608
Ssc.4978.3.A1_at	BF75	-0.404702025	2.45522E-05
Ssc.5014.1.S1_at	IMF	-0.348595562	0.000353618
Ssc.5021.1.S1_at	BF75	-0.431301965	6.02189E-06
Ssc.5028.1.A1_at	BFS	-0.322555415	0.000945233
Ssc.5096.1.A1_at	BF75	0.339255052	0.000486419
Ssc.5121.1.S1_at	HW	0.441552366	4.76234E-06
Ssc.5137.1.S1_at	BF75	-0.493281077	1.383E-07
Ssc.5480.1.S1_at	BW150	0.35263288	0.000343848
Ssc.5543.1.A1_at	BFS	-0.394189543	4.14361E-05
Ssc.5602.1.A1_at	BF75	-0.346320856	0.000363071
Ssc.5870.2.A1_at	BF75	-0.344266928	0.000395569
Ssc.5891.2.S1_a_at	BF75	-0.364975056	0.000162198
Ssc.5924.1.S1_at	HW	0.342813958	0.000513686
Ssc.5981.1.S1_at	LBW	0.364508651	0.0002079
Ssc.6024.3.S1_at	BF75	-0.334189445	0.000597376
Ssc.6035.1.S1_at	BW150	-0.356962563	0.000286869
Ssc.6044.1.S1_at	IMF	-0.340110326	0.000501667
Ssc.6056.1.S1_at	BF75	-0.499751335	8.92036E-08
Ssc.6092.3.S1_at	BFS	-0.33838799	0.000503955
Ssc.6186.1.A1_at	BFS	-0.365881287	0.000155773
Ssc.6193.1.A1_at	HW	0.348889734	0.000401321
Ssc.6209.1.A1_at	BFS	-0.459641939	1.17533E-06
Ssc.6237.1.S1_at	HW	0.362990132	0.000221958
Ssc.6315.1.S1_at	BFS	-0.366735127	0.000149937
Ssc.6341.3.A1_at	BFS	-0.334310525	0.000594474
Ssc.6373.1.A1_at	HW	0.39433735	5.37468E-05
Ssc.6381.1.S1_at	HW	0.334112586	0.000725295
Ssc.6447.1.A1_at	IMF	-0.337566182	0.000556066

Ssc.6511.1.A1_at	HW	-0.339708676	0.00058165
Ssc.6529.1.A1_at	HW	-0.460637738	1.60541E-06
Ssc.6529.2.S1_at	HW	-0.432308515	7.87942E-06
Ssc.6613.1.A1_at	BF75	-0.345804833	0.000370996
Ssc.6634.2.S1_at	IMF	-0.340102512	0.000501827
Ssc.6646.1.S1_at	BFS	-0.354974194	0.000251378
Ssc.6683.1.S1_at	BW150	0.335342739	0.00069119
Ssc.6779.1.S1_at	BW150	-0.584498548	2.12858E-10
Ssc.6807.1.A1_at	BFS	-0.321124151	0.000998895
Ssc.6811.1.A1_at	HW	-0.40972393	2.53838E-05
Ssc.6811.2.S1_at	HW	-0.408572552	2.6884E-05
Ssc.6877.1.A1_at	BF75	-0.383046261	7.08061E-05
Ssc.6896.1.A1_at	BF75	-0.355384028	0.000246974
Ssc.6909.1.A1_at	HW	-0.333099424	0.000754533
Ssc.70.1.S1_at	BF75	-0.392349951	4.53284E-05
Ssc.7060.2.S1_at	HW	-0.403253671	3.49552E-05
Ssc.7107.1.A1_at	LBW	-0.335683226	0.000682013
Ssc.7112.1.A1_at	BFS	-0.32549061	0.000843325
Ssc.7163.1.A1_at	BFS	-0.323494977	0.000911451
Ssc.7179.2.S1_a_at	BW150	0.380196695	0.000103735
Ssc.719.2.A1_at	BF75	-0.337433298	0.000523935
Ssc.7190.1.S1_at	BW150	-0.523741431	2.65436E-08
Ssc.7209.1.A1_at	IMF	0.325297502	0.000902538
Ssc.7225.1.A1_at	BW150	0.343095431	0.000507901
Ssc.7225.2.S1_at	HW	0.332756152	0.00076468
Ssc.7332.1.A1_at	BFS	-0.337408022	0.000524473
Ssc.734.1.S1_at	BFS	-0.358631303	0.00021452
Ssc.7400.1.A1_at	BF75	-0.350075552	0.000309937
Ssc.7413.1.A1_at	BW150	-0.530511938	1.62384E-08
Ssc.7422.1.A1_at	HW	-0.374595031	0.000133506
Ssc.7426.1.A1_at	BF75	-0.406062264	2.29149E-05
Ssc.7517.1.A1_at	BW150	-0.454079795	2.35004E-06
Ssc.7524.1.A1_at	BF75	-0.321842642	0.000971619
Ssc.7613.1.A1_at	LBW	-0.328933556	0.000886421
Ssc.7666.1.A1_at	BW150	-0.349104857	0.000397792
Ssc.7685.1.A1_at	BFS	-0.378672602	8.69238E-05
Ssc.7711.1.A1_at	BFS	-0.323082643	0.000926139
Ssc.7713.1.A1_at	BFS	0.387680816	5.67945E-05
Ssc.7724.1.A1_at	IMF	-0.350651133	0.000324409
Ssc.7739.1.A1_at	HW	-0.336428127	0.000662323
Ssc.7750.2.A1_at	BF75	-0.365160277	0.000160865
Ssc.7774.1.A1_at	BFS	-0.359167442	0.000209558
Ssc.7800.1.A1_at	BFS	-0.353026326	0.000273317
Ssc.7835.1.A1_at	HW	-0.331914537	0.00079009
Ssc.7850.2.A1_at	BW150	-0.414710039	1.97471E-05
Ssc.7862.1.A1_at	BFS	-0.34698841	0.00035305
Ssc.7866.1.A1_at	HW	-0.334277144	0.000720645
Ssc.7894.1.A1_at	BW150	-0.485176246	3.58885E-07
Ssc.7917.1.A1_at	BFS	-0.338698572	0.000497608
Ssc.8058.1.A1_at	HW	-0.42331676	1.26808E-05
Ssc.8078.1.A1_at	LBW	-0.340528671	0.000562945
Ssc.8096.1.A1_at	BFS	-0.361923685	0.00018568
Ssc.8202.1.S1_at	BFS	-0.335177151	0.000574072
Ssc.8213.1.A1_at	BF75	-0.329383589	0.000723637
Ssc.8213.2.A1_at	BF75	-0.36990118	0.000130018
Ssc.824.1.S1_at	BFS	-0.380696083	7.90839E-05
Ssc.8270.1.S1_at	HW	-0.326575378	0.00097009
Ssc.8317.1.A1_at	HW	0.353005769	0.000338558
Ssc.8347.1.A1_at	BFS	0.369000004	0.000135422
Ssc.8429.2.S1_at	BFS	-0.323577408	0.000908541
Ssc.8484.1.A1_at	BF75	-0.487316116	2.05561E-07
Ssc.8520.1.A1_at	BF75	-0.325706013	0.000836257
Ssc.8521.1.A1_at	BF75	-0.358406919	0.000216629
Ssc.8679.1.A1_at	BF75	-0.321459548	0.000986076
Ssc.8680.1.A1_at	BF75	-0.340120459	0.000469476
Ssc.873.1.S1_at	BW150	-0.367310965	0.000184096

Ssc.8808.1.A1_at	BFS	-0.419332799	1.15021E-05
Ssc.8821.2.A1_at	BF75	-0.414441628	1.48793E-05
Ssc.8837.1.A1_at	BFS	-0.338245898	0.000506884
Ssc.889.1.A1_a_at	IMF	-0.327416121	0.000831304
Ssc.8901.1.A1_at	IMF	-0.334510459	0.000628532
Ssc.894.1.A1_at	HW	-0.339948774	0.000576115
Ssc.9004.1.A1_at	BF75	-0.347605298	0.000344017
Ssc.9052.1.S1_at	HW	0.358243973	0.000271758
Ssc.9109.1.A1_at	HW	-0.37507302	0.000130686
Ssc.9160.1.A1_at	BFS	-0.342126957	0.000432258
Ssc.9220.1.A1_at	BFS	-0.328420678	0.000751699
Ssc.9235.1.A1_at	BW150	-0.32891492	0.000887056
Ssc.9244.1.A1_at	LBW	-0.327660627	0.000930729
Ssc.932.1.A1_at	BF75	-0.442192491	3.27016E-06
Ssc.9354.1.S1_at	BF75	-0.335571458	0.000565004
Ssc.9365.2.S1_a_at	HW	0.440477615	5.0533E-06
Ssc.9365.3.S1_a_at	HW	0.394847599	5.2457E-05
Ssc.9365.3.S1_x_at	HW	0.393156453	5.68461E-05
Ssc.9365.5.S1_a_at	HW	0.408306123	2.72428E-05
Ssc.9365.5.S1_at	BF75	-0.34126402	0.000447919
Ssc.9365.6.S1_x_at	HW	0.387650233	7.3624E-05
Ssc.9387.2.S1_at	BW150	-0.499873694	1.38128E-07
Ssc.9479.1.A1_at	BFS	-0.328155522	0.0007596
Ssc.9521.1.A1_at	HW	-0.357072623	0.000285541
Ssc.9573.1.A1_at	BFS	-0.336911452	0.00053516
Ssc.9630.1.A1_at	HW	0.332944364	0.000759101
Ssc.9659.1.A1_at	BF75	-0.373874952	0.000108493
Ssc.9672.2.S1_at	IMF	-0.360847269	0.000209694
Ssc.9684.2.A1_at	BF75	-0.438203214	4.09982E-06
Ssc.9715.1.A1_at	HW	-0.410723622	2.41452E-05
Ssc.9741.1.A1_at	HW	-0.338262807	0.000616026
Ssc.9784.1.S1_at	BF75	-0.340062367	0.000470596
Ssc.979.1.S1_at	HW	0.337260577	0.000640936
Ssc.9812.1.A1_at	BFS	-0.366863978	0.000149074
Ssc.9916.1.S1_at	HW	-0.478476954	5.46557E-07
Ssc.9951.1.A1_at	IMF	0.372880554	0.000122925
SscAffx.21.1.S1_at	BFS	-0.388877153	5.36232E-05

ARTÍCULO 2: Deciphering the regulation of porcine genes influencing growth, fatness and yield-related traits through genetical genomics.

Supplemental Table 3: Whole results of the eGWAS.

Probeset	SNP	Chromosome	Position	A1	A2	effB	se_effB	Pval	QVal
Ssc.10298.1.A1_at	ASGA0037721	0	0	G	C	-0.3631	0.0829	7.27E-06	9.99E-03
Ssc.10298.1.A1_at	ASGA0104632	0	0	A	G	-0.3265	0.0798	2.77E-05	2.79E-02
Ssc.10589.1.A1_at	ALGA0070432	0	0	C	A	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	ALGA0108193	0	0	G	A	-0.2211	0.0370	3.90E-06	5.36E-03
Ssc.10589.1.A1_at	ALGA0123845	0	0	A	G	-0.1721	0.0339	8.84E-05	2.54E-02
Ssc.10589.1.A1_at	ASGA0104596	0	0	G	A	-0.1772	0.0337	4.79E-05	1.58E-02
Ssc.10589.1.A1_at	DIAS0004226	0	0	G	A	0.1919	0.0394	1.66E-04	3.94E-02
Ssc.10589.1.A1_at	H3GA0037114	0	0	G	A	-0.1626	0.0331	1.47E-04	3.61E-02
Ssc.10589.1.A1_at	H3GA0055518	0	0	G	A	-0.2133	0.0343	1.47E-06	3.32E-03
Ssc.10589.1.A1_at	MARC0000700	0	0	A	G	-0.2211	0.0370	3.90E-06	5.36E-03
Ssc.10589.1.A1_at	MARC0043642	0	0	A	G	-0.2211	0.0370	3.90E-06	5.36E-03
Ssc.10589.1.A1_at	MARC0103691	0	0	A	C	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10952.1.S1_at	ALGA0015157	0	0	C	A	0.2468	0.0508	5.14E-07	2.01E-03
Ssc.11158.2.S1_at	MARC0043542	0	0	G	A	-0.3343	0.0528	1.38E-07	2.12E-03
Ssc.11208.1.S1_at	ASGA0014315	0	0	A	G	-0.3827	0.0880	3.04E-06	6.40E-03
Ssc.15678.1.A1_s_at	ALGA0106430	0	0	G	A	-0.4894	0.1119	2.91E-06	4.19E-03
Ssc.15678.1.A1_s_at	ASGA0089941	0	0	A	G	0.5529	0.1307	6.05E-06	7.09E-03
Ssc.15678.1.A1_s_at	H3GA0023693	0	0	G	A	-0.4894	0.1119	2.91E-06	4.19E-03
Ssc.15678.1.A1_s_at	H3GA0053374	0	0	A	G	0.4874	0.1028	3.97E-07	8.36E-04
Ssc.15678.1.A1_s_at	MARC0100327	0	0	A	G	0.4874	0.1028	3.97E-07	8.36E-04
Ssc.1860.1.S1_at	ALGA0105316	0	0	A	G	0.1627	0.0349	3.27E-07	1.15E-03
Ssc.1860.1.S1_at	ALGA0110504	0	0	G	A	-0.1308	0.0339	2.40E-05	4.74E-02
Ssc.1860.1.S1_at	ASGA0083835	0	0	A	C	0.1849	0.0386	1.49E-07	6.71E-04
Ssc.1860.1.S1_at	ASGA0097914	0	0	G	A	0.2003	0.0364	1.56E-09	1.65E-05
Ssc.18795.1.A1_at	MARC0029879	0	0	A	G	0.0794	0.0188	2.63E-05	4.18E-02
Ssc.19558.1.S1_at	ASGA0085325	0	0	G	A	0.4042	0.0806	6.40E-07	1.97E-02
Ssc.19634.1.S1_at	ALGA0040516	0	0	C	A	-0.2249	0.0498	1.04E-05	2.74E-02
Ssc.19634.1.S1_at	H3GA0020971	0	0	A	C	-0.2648	0.0604	1.86E-05	3.67E-02
Ssc.20525.1.S1_at	ALGA0011405	0	0	A	C	-0.1450	0.0360	4.04E-06	1.28E-02
Ssc.20525.1.S1_at	ASGA0085597	0	0	A	G	0.2081	0.0482	7.54E-07	2.98E-03
Ssc.20525.1.S1_at	ASGA0086466	0	0	A	G	0.1457	0.0367	5.41E-06	1.56E-02
Ssc.20525.1.S1_at	ASGA0095982	0	0	G	A	0.2081	0.0482	7.54E-07	2.98E-03
Ssc.20525.1.S1_at	ASGA0096364	0	0	A	G	0.2081	0.0482	7.54E-07	2.98E-03
Ssc.20525.1.S1_at	ASGA0105167	0	0	G	A	0.3164	0.0634	1.07E-08	3.39E-04
Ssc.20525.1.S1_at	H3GA0005551	0	0	G	A	-0.1999	0.0521	1.09E-05	2.46E-02
Ssc.20525.1.S1_at	H3GA0054053	0	0	A	G	0.2081	0.0482	7.54E-07	2.98E-03
Ssc.20525.1.S1_at	M1GA0027267	0	0	A	G	-0.3170	0.0687	1.26E-07	1.33E-03
Ssc.20525.1.S1_at	MARC0051258	0	0	A	G	0.1800	0.0489	2.48E-05	3.91E-02
Ssc.21242.1.S1_at	ALGA0095121	0	0	A	G	-0.1692	0.0378	5.11E-06	1.61E-02
Ssc.21242.1.S1_at	ALGA0095458	0	0	A	G	0.1683	0.0392	1.20E-05	2.37E-02
Ssc.21242.1.S1_at	ALGA0107226	0	0	G	A	0.1853	0.0392	1.42E-06	8.31E-03
Ssc.2152.1.A1_at	ALGA0117732	0	0	A	G	-0.1039	0.0206	9.81E-08	9.69E-05
Ssc.2152.1.A1_at	ASGA0090023	0	0	A	G	-0.0983	0.0208	6.05E-07	5.63E-04
Ssc.2152.1.A1_at	ASGA0104810	0	0	G	A	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	DIAS0000443	0	0	G	A	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	DIAS0003496	0	0	A	G	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	MARC0015595	0	0	G	A	-0.0983	0.0208	6.05E-07	5.63E-04
Ssc.21861.1.S1_at	ALGA0076768	0	0	C	G	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	MARC0022133	0	0	A	G	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	MARC0083561	0	0	G	A	0.4171	0.0992	1.64E-05	1.72E-02

Ssc.21969.1.A1_at	ASGA0083721	0	0	C	A	0.1675	0.0321	3.26E-09	1.72E-05
Ssc.25555.1.S1_at	ALGA0119668	0	0	A	G	-0.3715	0.0734	7.53E-07	3.68E-03
Ssc.25555.1.S1_at	MARC0070281	0	0	G	A	-0.3715	0.0734	7.53E-07	3.68E-03
Ssc.30633.1.S1_at	ALGA0116674	0	0	C	A	-0.2589	0.0459	7.31E-07	5.88E-03
Ssc.30633.1.S1_at	ASGA0093255	0	0	G	A	-0.2589	0.0459	7.31E-07	5.88E-03
Ssc.30987.1.S1_at	DRGA0014612	0	0	A	G	-0.0727	0.0166	6.79E-08	7.15E-04
Ssc.7666.1.A1_at	ALGA0095458	0	0	A	G	0.1829	0.0412	2.45E-05	2.68E-02
Ssc.7666.1.A1_at	ALGA0107226	0	0	G	A	0.2092	0.0412	1.37E-06	1.08E-02
Ssc.7666.1.A1_at	ALGA0113098	0	0	A	G	0.1741	0.0401	3.63E-05	3.23E-02
Ssc.7666.1.A1_at	ASGA0075579	0	0	A	G	-0.1879	0.0415	1.68E-05	2.05E-02
Ssc.7666.1.A1_at	ASGA0095017	0	0	G	A	-0.1804	0.0419	4.19E-05	3.23E-02
Ssc.9109.1.A1_at	ALGA0080719	0	0	G	A	0.0188	0.0043	1.71E-05	1.26E-02
Ssc.9109.1.A1_at	DRGA0014278	0	0	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	INRA0046035	0	0	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0014154	0	0	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0051655	0	0	G	A	-0.0195	0.0048	6.38E-05	1.36E-02
Ssc.9365.2.S1_a_at	ALGA0011405	0	0	A	C	-0.2326	0.0544	1.49E-06	4.70E-03
Ssc.9365.2.S1_a_at	ASGA0008471	0	0	A	G	-0.2283	0.0629	4.35E-05	3.20E-02
Ssc.9365.2.S1_a_at	ASGA0085597	0	0	A	G	0.3486	0.0727	6.84E-08	3.09E-04
Ssc.9365.2.S1_a_at	ASGA0086466	0	0	A	G	0.2262	0.0554	4.27E-06	6.43E-03
Ssc.9365.2.S1_a_at	ASGA0095646	0	0	A	C	0.2540	0.0617	3.58E-06	6.29E-03
Ssc.9365.2.S1_a_at	ASGA0095982	0	0	G	A	0.3486	0.0727	6.84E-08	3.09E-04
Ssc.9365.2.S1_a_at	ASGA0096364	0	0	A	G	0.3486	0.0727	6.84E-08	3.09E-04
Ssc.9365.2.S1_a_at	ASGA0105167	0	0	G	A	0.5336	0.0957	3.41E-10	1.08E-05
Ssc.9365.2.S1_a_at	H3GA0005551	0	0	G	A	-0.3140	0.0786	6.82E-06	8.98E-03
Ssc.9365.2.S1_a_at	H3GA0054053	0	0	A	G	0.3486	0.0727	6.84E-08	3.09E-04
Ssc.9365.2.S1_a_at	M1GA0027267	0	0	A	G	-0.5419	0.1037	4.05E-09	6.41E-05
Ssc.9365.2.S1_a_at	MARC0051258	0	0	A	G	0.3025	0.0738	3.95E-06	6.43E-03
Ssc.9916.1.S1_at	ASGA0049832	0	0	G	A	-0.0807	0.0154	1.89E-08	1.99E-04
Ssc.9916.1.S1_at	ASGA0104249	0	0	A	G	-0.1167	0.0303	3.63E-05	4.14E-02
Ssc.1860.1.S1_at	ASGA0000712	1	8,988,180	A	G	0.1761	0.0374	2.38E-07	9.39E-04
Ssc.1860.1.S1_at	M1GA0000623	1	9,221,985	G	A	-0.1393	0.0328	3.25E-06	7.89E-03
Ssc.1860.1.S1_at	DRGA0000068	1	10,233,204	A	G	0.2077	0.0429	1.10E-07	5.81E-04
Ssc.1860.1.S1_at	ALGA0000773	1	10,275,177	A	G	-0.1881	0.0338	1.10E-09	1.65E-05
Ssc.1860.1.S1_at	ASGA0000856	1	10,393,512	A	G	-0.1881	0.0338	1.10E-09	1.65E-05
Ssc.1860.1.S1_at	H3GA0000689	1	10,483,398	A	G	0.1731	0.0418	5.65E-06	1.28E-02
Ssc.1860.1.S1_at	ALGA0106880	1	11,762,368	G	A	0.1816	0.0355	2.12E-08	1.67E-04
Ssc.1860.1.S1_at	MARC0008026	1	14,038,761	G	A	0.1815	0.0373	9.57E-08	5.81E-04
Ssc.1860.1.S1_at	ALGA0001109	1	14,874,949	C	A	0.1860	0.0435	2.75E-06	7.24E-03
Ssc.1860.1.S1_at	ALGA0001100	1	15,078,292	C	A	0.1860	0.0435	2.75E-06	7.24E-03
Ssc.1860.1.S1_at	DRGA0000110	1	15,231,294	A	G	0.1879	0.0433	2.00E-06	6.31E-03
Ssc.25573.1.S1_at	ALGA0005664	1	129,136,194	A	G	-0.1201	0.0306	1.07E-05	3.76E-02
Ssc.25573.1.S1_at	MARC0072090	1	129,160,216	A	C	-0.1201	0.0306	1.07E-05	3.76E-02
Ssc.25573.1.S1_at	ALGA0109145	1	129,268,324	G	A	-0.1201	0.0306	1.07E-05	3.76E-02
Ssc.25573.1.S1_at	ALGA0005676	1	129,316,644	A	C	-0.1201	0.0306	1.07E-05	3.76E-02
Ssc.25573.1.S1_at	H3GA0002645	1	130,108,758	G	A	-0.1201	0.0306	1.07E-05	3.76E-02
Ssc.25573.1.S1_at	ALGA0005701	1	130,121,507	G	A	-0.1201	0.0306	1.07E-05	3.76E-02
Ssc.25573.1.S1_at	MARC0007790	1	130,163,429	G	A	-0.1201	0.0306	1.07E-05	3.76E-02
Ssc.25573.1.S1_at	MARC0021068	1	130,167,976	G	A	-0.1201	0.0306	1.07E-05	3.76E-02
Ssc.20685.1.S1_at	ALGA0006009	1	146,180,618	G	A	0.0456	0.0107	1.25E-06	7.89E-03
Ssc.20685.1.S1_at	INRA0004226	1	146,216,178	G	A	0.0456	0.0107	1.25E-06	7.89E-03
Ssc.20685.1.S1_at	DIAS0000131	1	146,247,253	G	A	0.0456	0.0107	1.25E-06	7.89E-03
Ssc.20685.1.S1_at	DIAS0003570	1	146,309,756	A	G	0.0456	0.0107	1.25E-06	7.89E-03
Ssc.20685.1.S1_at	ASGA0004564	1	146,332,008	G	A	0.0456	0.0107	1.25E-06	7.89E-03
Ssc.25573.1.S1_at	ALGA0006302	1	162,259,035	A	G	0.1307	0.0323	5.70E-06	3.76E-02
Ssc.30633.1.S1_at	ALGA0106630	1	309,039,258	G	A	-0.2572	0.0461	9.30E-07	5.88E-03
Ssc.30633.1.S1_at	ALGA0106999	1	309,125,803	G	A	-0.2572	0.0461	9.30E-07	5.88E-03
Ssc.9365.2.S1_a_at	ASGA0084177	2	16,416	G	A	0.2540	0.0617	3.58E-06	6.29E-03

Ssc.20525.1.S1_at	ALGA0110785	2	251,447	G	A	0.1499	0.0406	2.39E-05	3.91E-02
Ssc.9365.2.S1_a_at	ALGA0110785	2	251,447	G	A	0.2588	0.0613	2.05E-06	4.98E-03
Ssc.20525.1.S1_at	ASGA0097367	2	365,309	A	G	0.1499	0.0406	2.39E-05	3.91E-02
Ssc.9365.2.S1_a_at	ASGA0097367	2	365,309	A	G	0.2588	0.0613	2.05E-06	4.98E-03
Ssc.20525.1.S1_at	MARC0022036	2	963,130	A	C	-0.1518	0.0424	4.13E-05	4.13E-02
Ssc.9365.2.S1_a_at	MARC0022036	2	963,130	A	C	-0.2636	0.0640	3.58E-06	6.29E-03
Ssc.20525.1.S1_at	MARC0008125	2	1,264,220	A	G	0.1448	0.0399	3.23E-05	4.09E-02
Ssc.9365.2.S1_a_at	MARC0008125	2	1,264,220	A	G	0.2253	0.0602	2.57E-05	2.20E-02
Ssc.20525.1.S1_at	ASGA0082213	2	1,457,259	A	C	0.1448	0.0399	3.23E-05	4.09E-02
Ssc.9365.2.S1_a_at	ASGA0082213	2	1,457,259	A	C	0.2253	0.0602	2.57E-05	2.20E-02
Ssc.20525.1.S1_at	ASGA0083230	2	1,460,039	A	G	0.1858	0.0440	1.30E-06	4.58E-03
Ssc.9365.2.S1_a_at	ASGA0083230	2	1,460,039	A	G	0.2961	0.0664	5.13E-07	2.03E-03
Ssc.20525.1.S1_at	ALGA0112642	2	1,505,302	A	G	0.1448	0.0399	3.23E-05	4.09E-02
Ssc.9365.2.S1_a_at	ALGA0112642	2	1,505,302	A	G	0.2253	0.0602	2.57E-05	2.20E-02
Ssc.20525.1.S1_at	ASGA0101159	2	2,224,107	G	A	-0.3240	0.0700	1.11E-07	1.33E-03
Ssc.9365.2.S1_a_at	ASGA0101159	2	2,224,107	G	A	-0.5415	0.1056	7.76E-09	8.17E-05
Ssc.20525.1.S1_at	INRA0008096	2	2,666,056	A	G	-0.1396	0.0380	2.60E-05	3.91E-02
Ssc.9365.2.S1_a_at	INRA0008096	2	2,666,056	A	G	-0.2206	0.0574	1.50E-05	1.58E-02
Ssc.20525.1.S1_at	ALGA0011460	2	3,606,836	G	A	0.1639	0.0456	3.87E-05	4.13E-02
Ssc.9365.2.S1_a_at	ALGA0011460	2	3,606,836	G	A	0.2610	0.0689	1.99E-05	1.90E-02
Ssc.20525.1.S1_at	H3GA0005630	2	3,788,156	A	G	0.1727	0.0446	9.05E-06	2.20E-02
Ssc.9365.2.S1_a_at	H3GA0005630	2	3,788,156	A	G	0.2749	0.0673	4.26E-06	6.43E-03
Ssc.20525.1.S1_at	H3GA0054097	2	4,402,567	G	A	0.1547	0.0409	1.48E-05	3.12E-02
Ssc.9365.2.S1_a_at	H3GA0054097	2	4,402,567	G	A	0.2384	0.0617	1.38E-05	1.51E-02
Ssc.20525.1.S1_at	MARC0089109	2	4,799,420	A	C	0.1403	0.0391	3.99E-05	4.13E-02
Ssc.9365.2.S1_a_at	MARC0089109	2	4,799,420	A	C	0.2197	0.0591	2.83E-05	2.29E-02
Ssc.9365.2.S1_a_at	ASGA0008604	2	5,148,429	A	G	0.2542	0.0688	3.21E-05	2.53E-02
Ssc.9365.2.S1_a_at	ASGA0008626	2	5,335,401	G	A	-0.3631	0.0872	2.79E-06	6.07E-03
Ssc.9365.2.S1_a_at	H3GA0055977	2	5,479,542	A	G	-0.2347	0.0595	9.04E-06	1.14E-02
Ssc.20525.1.S1_at	ASGA0008719	2	6,094,116	A	C	0.1357	0.0373	3.13E-05	4.09E-02
Ssc.9365.2.S1_a_at	ASGA0008719	2	6,094,116	A	C	0.2186	0.0562	1.18E-05	1.34E-02
Ssc.20525.1.S1_at	DIAS0000846	2	6,201,646	A	C	0.2585	0.0598	7.40E-07	2.98E-03
Ssc.9365.2.S1_a_at	DIAS0000846	2	6,201,646	A	C	0.3894	0.0903	1.20E-06	4.23E-03
Ssc.20525.1.S1_at	H3GA0005759	2	6,667,399	G	A	0.1815	0.0466	8.03E-06	2.11E-02
Ssc.9365.2.S1_a_at	H3GA0005759	2	6,667,399	G	A	0.2852	0.0703	4.94E-06	7.10E-03
Ssc.20525.1.S1_at	ASGA0084012	2	8,197,811	A	G	0.1626	0.0456	4.41E-05	4.13E-02
Ssc.9365.2.S1_a_at	ASGA0084012	2	8,197,811	A	G	0.2608	0.0688	1.99E-05	1.90E-02
Ssc.20525.1.S1_at	ASGA0097057	2	8,244,738	C	A	0.1626	0.0456	4.41E-05	4.13E-02
Ssc.9365.2.S1_a_at	ASGA0097057	2	8,244,738	C	A	0.2608	0.0688	1.99E-05	1.90E-02
Ssc.20525.1.S1_at	DIAS0003229	2	8,281,382	A	G	0.1421	0.0399	4.44E-05	4.13E-02
Ssc.9365.2.S1_a_at	DIAS0003229	2	8,281,382	A	G	0.2255	0.0602	2.47E-05	2.20E-02
Ssc.9365.2.S1_a_at	H3GA0052992	2	8,379,515	G	A	-0.1987	0.0556	5.85E-05	4.20E-02
Ssc.9365.2.S1_a_at	ASGA0102529	2	8,596,505	A	G	0.2972	0.0799	2.80E-05	2.29E-02
Ssc.20525.1.S1_at	MARC0035734	2	8,609,588	A	G	0.1385	0.0374	2.19E-05	3.91E-02
Ssc.9365.2.S1_a_at	MARC0035734	2	8,609,588	A	G	0.2197	0.0564	1.17E-05	1.34E-02
Ssc.9365.2.S1_a_at	MARC0018949	2	8,997,005	G	A	-0.2092	0.0536	1.10E-05	1.33E-02
Ssc.20525.1.S1_at	ALGA0112799	2	9,613,380	G	A	-0.2155	0.0600	3.86E-05	4.13E-02
Ssc.20525.1.S1_at	H3GA0005955	2	10,979,357	G	A	-0.1440	0.0402	4.01E-05	4.13E-02
Ssc.9365.2.S1_a_at	H3GA0005955	2	10,979,357	G	A	-0.2519	0.0606	2.88E-06	6.07E-03
Ssc.8901.1.A1_at	ALGA0105266	2	18,376,106	A	G	0.0627	0.0136	1.40E-06	3.56E-02
Ssc.10952.1.S1_at	H3GA0007378	2	118,359,766	A	G	-0.2486	0.0488	1.38E-07	1.82E-03
Ssc.10952.1.S1_at	ALGA0113999	2	119,322,376	C	A	0.2297	0.0505	2.57E-06	8.04E-03
Ssc.10952.1.S1_at	ASGA0089346	2	119,392,780	A	G	0.2385	0.0491	5.07E-07	2.01E-03
Ssc.10952.1.S1_at	ASGA0097507	2	119,465,826	G	A	0.2468	0.0508	5.14E-07	2.01E-03
Ssc.10952.1.S1_at	MARC0006600	2	119,475,782	A	G	0.2498	0.0504	2.90E-07	1.82E-03
Ssc.10952.1.S1_at	MARC0104000	2	119,497,356	A	G	0.2498	0.0504	2.90E-07	1.82E-03
Ssc.10952.1.S1_at	ASGA0011312	2	119,790,195	A	G	0.2498	0.0504	2.90E-07	1.82E-03
Ssc.10952.1.S1_at	MARC0052388	2	120,009,260	G	A	0.2353	0.0505	1.46E-06	5.08E-03

Ssc.10952.1.S1_at	MARC0066799	2	120,243,915	A	C	0.2511	0.0499	1.95E-07	1.82E-03
Ssc.20525.1.S1_at	ALGA0111915	2	162,084,552	G	A	0.1499	0.0406	2.39E-05	3.91E-02
Ssc.9365.2.S1_a_at	ALGA0111915	2	162,084,552	G	A	0.2588	0.0613	2.05E-06	4.98E-03
Ssc.20525.1.S1_at	ASGA0085784	2	162,298,086	G	A	0.1503	0.0419	3.94E-05	4.13E-02
Ssc.9365.2.S1_a_at	ASGA0085784	2	162,298,086	G	A	0.2549	0.0632	5.68E-06	7.81E-03
Ssc.29388.1.A1_at	ASGA0013555	3	14,221,604	A	G	-0.4867	0.1006	1.76E-06	2.73E-02
Ssc.29388.1.A1_at	MARC0046084	3	14,772,381	G	A	-0.4975	0.0991	7.05E-07	2.18E-02
Ssc.11208.1.S1_at	ALGA0018561	3	37,775,709	A	G	0.3567	0.0921	3.18E-05	4.48E-02
Ssc.11208.1.S1_at	ALGA0018634	3	45,593,990	A	G	-0.3011	0.0773	2.91E-05	4.38E-02
Ssc.11208.1.S1_at	ALGA0018858	3	49,542,819	A	G	0.3147	0.0813	3.26E-05	4.48E-02
Ssc.11208.1.S1_at	ASGA0014713	3	58,059,120	A	G	0.4269	0.0896	3.14E-07	9.79E-04
Ssc.11208.1.S1_at	MARC0008876	3	58,746,097	C	A	0.4269	0.0896	3.14E-07	9.79E-04
Ssc.11208.1.S1_at	ALGA0019165	3	58,756,395	G	A	0.4109	0.0894	7.92E-07	1.79E-03
Ssc.11208.1.S1_at	ASGA0103924	3	58,788,483	G	A	0.4109	0.0894	7.92E-07	1.79E-03
Ssc.11208.1.S1_at	ALGA0124343	3	58,788,742	A	C	0.4109	0.0894	7.92E-07	1.79E-03
Ssc.11208.1.S1_at	H3GA0009642	3	58,873,656	G	A	-0.3332	0.0847	2.39E-05	3.78E-02
Ssc.11208.1.S1_at	ALGA0019167	3	58,928,182	A	C	0.4569	0.0902	5.46E-08	8.64E-04
Ssc.11208.1.S1_at	ALGA0019175	3	59,150,121	A	G	0.4569	0.0902	5.46E-08	8.64E-04
Ssc.11208.1.S1_at	ALGA0019250	3	60,663,016	A	C	0.4333	0.0881	1.30E-07	9.79E-04
Ssc.11208.1.S1_at	MARC0032347	3	60,693,443	A	G	-0.3457	0.0875	2.24E-05	3.78E-02
Ssc.11208.1.S1_at	MARC0017363	3	60,864,474	A	G	0.4230	0.0891	3.41E-07	9.79E-04
Ssc.11208.1.S1_at	MARC0002058	3	61,482,786	A	G	0.4230	0.0891	3.41E-07	9.79E-04
Ssc.11208.1.S1_at	ALGA0019285	3	61,499,337	C	A	0.4230	0.0891	3.41E-07	9.79E-04
Ssc.11208.1.S1_at	M1GA0004410	3	61,556,324	A	G	0.4230	0.0891	3.41E-07	9.79E-04
Ssc.11208.1.S1_at	MARC0054644	3	61,588,892	G	A	0.4230	0.0891	3.41E-07	9.79E-04
Ssc.11208.1.S1_at	CASI0010035	3	61,616,860	A	G	0.4230	0.0891	3.41E-07	9.79E-04
Ssc.11208.1.S1_at	DIAS0001331	3	62,206,398	A	G	0.3601	0.0915	2.37E-05	3.78E-02
Ssc.11208.1.S1_at	ALGA0111328	3	62,275,498	G	A	0.3601	0.0915	2.37E-05	3.78E-02
Ssc.11208.1.S1_at	MARC0027326	3	62,507,762	G	A	0.3748	0.0907	9.25E-06	1.83E-02
Ssc.21242.1.S1_at	ALGA0106200	3	83,577,449	G	A	0.1450	0.0360	4.01E-05	4.65E-02
Ssc.7666.1.A1_at	ALGA0106200	3	83,577,449	G	A	0.1624	0.0379	4.51E-05	3.24E-02
Ssc.7060.2.S1_at	MARC0010914	3	93,221,408	G	A	-0.2037	0.0432	2.51E-06	3.96E-02
Ssc.7060.2.S1_at	ALGA0020040	3	94,469,016	G	A	-0.1842	0.0418	1.07E-05	4.21E-02
Ssc.7060.2.S1_at	ALGA0020036	3	94,536,421	C	A	-0.1842	0.0418	1.07E-05	4.21E-02
Ssc.7060.2.S1_at	ALGA0020047	3	95,247,199	G	A	-0.1842	0.0418	1.07E-05	4.21E-02
Ssc.7060.2.S1_at	ALGA0020052	3	95,447,725	G	A	-0.1842	0.0418	1.07E-05	4.21E-02
Ssc.7060.2.S1_at	H3GA0010041	3	95,860,345	A	G	-0.1842	0.0418	1.07E-05	4.21E-02
Ssc.7060.2.S1_at	ASGA0015286	3	95,890,890	A	G	-0.1842	0.0418	1.07E-05	4.21E-02
Ssc.7060.2.S1_at	H3GA0010049	3	97,347,171	A	G	-0.1810	0.0384	2.49E-06	3.96E-02
Ssc.26316.1.S1_at	DRGA0004159	3	111,541,225	G	A	-0.1862	0.0454	3.04E-06	8.72E-03
Ssc.26316.1.S1_at	ALGA0020596	3	111,760,147	A	C	-0.1862	0.0454	3.04E-06	8.72E-03
Ssc.26316.1.S1_at	ASGA0015717	3	111,796,709	A	G	-0.1862	0.0454	3.04E-06	8.72E-03
Ssc.26316.1.S1_at	ALGA0020603	3	111,986,113	A	G	-0.1862	0.0454	3.04E-06	8.72E-03
Ssc.26316.1.S1_at	ALGA0020605	3	112,022,721	G	A	-0.1862	0.0454	3.04E-06	8.72E-03
Ssc.26316.1.S1_at	ASGA0015724	3	112,051,649	A	G	-0.1862	0.0454	3.04E-06	8.72E-03
Ssc.26316.1.S1_at	MARC0076643	3	112,552,012	C	A	-0.1862	0.0454	3.04E-06	8.72E-03
Ssc.26316.1.S1_at	ALGA0020626	3	112,572,514	A	G	-0.1862	0.0454	3.04E-06	8.72E-03
Ssc.26316.1.S1_at	ALGA0020627	3	112,594,087	G	A	-0.1862	0.0454	3.04E-06	8.72E-03
Ssc.26316.1.S1_at	ASGA0015742	3	112,674,884	A	G	-0.1862	0.0454	3.04E-06	8.72E-03
Ssc.26316.1.S1_at	ASGA0015738	3	112,768,775	G	A	-0.1862	0.0454	3.04E-06	8.72E-03
Ssc.25555.1.S1_at	ASGA0018396	4	13,084,536	A	C	-0.3733	0.0739	8.14E-07	3.68E-03
Ssc.25555.1.S1_at	ASGA0018418	4	13,330,048	G	A	0.3813	0.0717	2.02E-07	2.13E-03
Ssc.25555.1.S1_at	MARC0057481	4	15,848,929	G	A	-0.3949	0.0767	4.91E-07	3.68E-03
Ssc.25555.1.S1_at	ASGA0093554	4	16,069,475	A	G	-0.3190	0.0698	8.15E-06	2.19E-02
Ssc.25555.1.S1_at	ASGA0090485	4	16,070,229	G	A	-0.3190	0.0698	8.15E-06	2.19E-02
Ssc.25555.1.S1_at	ALGA0114568	4	16,347,684	A	G	0.3256	0.0708	6.98E-06	2.19E-02
Ssc.25555.1.S1_at	DIAS0000258	4	19,818,145	G	A	0.3927	0.0718	9.20E-08	1.45E-03
Ssc.25555.1.S1_at	MARC0061914	4	20,675,792	G	A	-0.3439	0.0770	1.30E-05	2.58E-02

Ssc.25555.1.S1_at	ASGA0092756	4	20,794,358	G	A	-0.3439	0.0770	1.30E-05	2.58E-02
Ssc.25555.1.S1_at	ASGA0018906	4	20,831,759	C	A	-0.3439	0.0770	1.30E-05	2.58E-02
Ssc.25555.1.S1_at	ALGA0023872	4	20,872,493	A	C	-0.4022	0.0729	7.06E-08	1.45E-03
Ssc.25555.1.S1_at	MARC0022418	4	21,180,738	A	G	0.3542	0.0747	3.59E-06	1.42E-02
Ssc.25555.1.S1_at	ALGA0023882	4	21,217,994	G	A	0.3296	0.0748	1.69E-05	2.81E-02
Ssc.25555.1.S1_at	ALGA0023888	4	21,255,882	A	G	0.3296	0.0748	1.69E-05	2.81E-02
Ssc.25555.1.S1_at	ASGA0018921	4	21,272,927	A	G	-0.2900	0.0683	3.39E-05	4.66E-02
Ssc.25555.1.S1_at	ALGA0023910	4	21,466,415	A	G	-0.3049	0.0692	1.69E-05	2.81E-02
Ssc.25555.1.S1_at	ALGA0023916	4	21,567,751	A	G	-0.3206	0.0746	2.69E-05	3.86E-02
Ssc.25555.1.S1_at	ALGA0023947	4	21,762,434	G	A	-0.3034	0.0700	2.30E-05	3.61E-02
Ssc.25555.1.S1_at	ALGA0116097	4	22,561,896	G	A	0.3344	0.0733	8.31E-06	2.19E-02
Ssc.25555.1.S1_at	MARC0009479	4	28,899,542	C	A	0.3069	0.0710	2.40E-05	3.61E-02
Ssc.25555.1.S1_at	ALGA0024196	4	29,558,810	G	A	0.3056	0.0676	9.93E-06	2.41E-02
Ssc.9109.1.A1_at	INRA0046679	4	63,841,367	A	C	0.0196	0.0048	6.28E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0049861	4	63,988,841	A	G	0.0196	0.0048	6.28E-05	1.36E-02
Ssc.18795.1.A1_at	MARC0007420	4	98,671,182	G	A	0.0794	0.0188	2.63E-05	4.18E-02
Ssc.7190.1.S1_at	H3GA0014377	4	128,983,125	A	G	0.1415	0.0356	4.50E-06	2.01E-02
Ssc.7190.1.S1_at	ALGA0028623	4	129,016,956	G	A	0.1415	0.0356	4.50E-06	2.01E-02
Ssc.1790.1.S1_at	ASGA0023685	5	876,762	A	G	0.1632	0.0314	1.10E-07	5.50E-04
Ssc.1790.1.S1_at	MARC0012115	5	921,586	A	G	0.1939	0.0336	3.67E-09	5.34E-05
Ssc.1790.1.S1_at	MARC0103593	5	961,240	G	A	0.1632	0.0314	1.10E-07	5.50E-04
Ssc.1790.1.S1_at	ASGA0081890	5	1,007,763	A	C	0.1658	0.0321	1.30E-07	5.57E-04
Ssc.1790.1.S1_at	ASGA0106073	5	1,046,890	G	A	0.1951	0.0345	7.13E-09	5.34E-05
Ssc.1790.1.S1_at	H3GA0015085	5	1,113,286	A	G	0.1480	0.0324	3.11E-06	1.17E-02
Ssc.1790.1.S1_at	MARC0068654	5	1,165,304	G	A	0.1951	0.0345	7.13E-09	5.34E-05
Ssc.1790.1.S1_at	INRA0018060	5	1,198,942	A	G	0.1957	0.0345	6.69E-09	5.34E-05
Ssc.1790.1.S1_at	MARC0010563	5	1,864,156	A	C	0.2005	0.0473	1.45E-05	4.83E-02
Ssc.18795.1.A1_at	H3GA0053864	6	48,585,961	A	G	0.0794	0.0188	2.63E-05	4.18E-02
Ssc.18795.1.A1_at	ALGA0102689	6	48,717,238	A	G	0.0794	0.0188	2.63E-05	4.18E-02
Ssc.18795.1.A1_at	ASGA0104037	6	48,792,292	A	G	0.0794	0.0188	2.63E-05	4.18E-02
Ssc.18795.1.A1_at	ASGA0089838	6	49,146,524	G	A	0.0794	0.0188	2.63E-05	4.18E-02
Ssc.18795.1.A1_at	ASGA0093393	6	49,168,322	G	A	0.0794	0.0188	2.63E-05	4.18E-02
Ssc.18795.1.A1_at	DIAS0000492	6	49,802,217	A	G	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	MARC0011519	6	49,817,264	A	G	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	H3GA0056470	6	50,006,716	G	A	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	DIAS0004447	6	50,037,571	A	G	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	DIAS0003231	6	50,065,951	A	G	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	MARC0032442	6	50,259,057	A	C	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	DIAS0000822	6	50,264,414	A	G	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	H3GA0053555	6	50,307,537	A	G	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	DIAS0003830	6	50,339,827	G	A	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	MARC0049189	6	50,364,492	G	A	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.894.1.A1_at	MARC0044346	6	50,478,565	A	C	0.2463	0.0531	2.20E-06	2.23E-02
Ssc.894.1.A1_at	M1GA0008536	6	50,495,796	G	A	0.2463	0.0531	2.20E-06	2.23E-02
Ssc.18795.1.A1_at	ASGA0028206	6	50,556,192	G	A	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	M1GA0008527	6	50,606,084	C	A	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	ASGA0028211	6	50,803,585	G	A	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	M1GA0008539	6	50,847,065	G	A	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	ASGA0103898	6	50,867,656	G	A	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.894.1.A1_at	MARC0098482	6	50,888,554	A	G	0.2463	0.0531	2.20E-06	2.23E-02
Ssc.18795.1.A1_at	ALGA0122867	6	51,104,922	G	A	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	ALGA0116613	6	51,139,647	A	G	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	ASGA0103416	6	51,352,837	A	G	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.18795.1.A1_at	ALGA0035323	6	51,611,976	A	G	0.0794	0.0188	2.63E-05	4.18E-02
Ssc.18795.1.A1_at	ALGA0035324	6	51,636,474	G	A	0.0794	0.0188	2.63E-05	4.18E-02
Ssc.18795.1.A1_at	ALGA0035318	6	51,678,926	G	A	0.0794	0.0188	2.63E-05	4.18E-02
Ssc.18795.1.A1_at	ASGA0028223	6	51,757,391	A	C	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.30633.1.S1_at	ALGA0035326	6	51,775,907	A	G	-0.2392	0.0442	2.04E-06	7.17E-03

Ssc.30633.1.S1_at	ASGA0028228	6	51,805,308	A	G	-0.2392	0.0442	2.04E-06	7.17E-03
Ssc.30633.1.S1_at	ALGA0035330	6	51,843,873	A	G	-0.2392	0.0442	2.04E-06	7.17E-03
Ssc.30633.1.S1_at	ALGA0115158	6	52,085,979	C	A	-0.2392	0.0442	2.04E-06	7.17E-03
Ssc.18795.1.A1_at	ASGA0097167	6	52,127,558	G	A	0.0794	0.0188	2.63E-05	4.18E-02
Ssc.18795.1.A1_at	ALGA0112704	6	52,226,606	G	A	0.0794	0.0188	2.63E-05	4.18E-02
Ssc.30633.1.S1_at	MARC0005462	6	52,262,806	G	A	-0.2415	0.0474	7.63E-06	2.41E-02
Ssc.18795.1.A1_at	MARC0049139	6	52,336,598	A	G	0.0731	0.0178	4.38E-05	4.18E-02
Ssc.30633.1.S1_at	H3GA0017980	6	52,993,696	G	A	-0.2589	0.0459	7.31E-07	5.88E-03
Ssc.2152.1.A1_at	ASGA0083551	6	80,520,240	G	A	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	ASGA0082468	6	80,819,138	A	G	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	H3GA0056112	6	81,021,081	A	C	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	H3GA0052514	6	81,135,210	A	G	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	MARC0016269	6	81,252,063	A	G	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	ALGA0116951	6	81,724,841	G	A	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	M1GA0008796	6	81,987,568	G	A	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	ALGA0035880	6	82,014,983	G	A	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	H3GA0052531	6	82,048,353	A	G	-0.1092	0.0211	4.85E-08	5.89E-05
Ssc.2152.1.A1_at	ASGA0028827	6	82,154,965	A	G	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	ASGA0028821	6	82,169,395	G	A	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.11158.2.S1_at	ASGA0028841	6	82,315,974	G	A	-0.3343	0.0528	1.38E-07	2.12E-03
Ssc.2152.1.A1_at	ALGA0114962	6	82,674,029	G	A	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	ALGA0113169	6	82,674,394	A	G	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	ASGA0100549	6	82,740,820	A	G	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	ASGA0091706	6	82,741,224	G	A	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	ALGA0123322	6	82,756,988	A	G	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	MARC0026717	6	82,765,130	G	A	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	MARC0098528	6	82,822,642	G	A	-0.1055	0.0204	4.68E-08	5.89E-05
Ssc.2152.1.A1_at	ALGA0117523	6	82,830,669	G	A	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	ALGA0115329	6	82,916,168	G	A	-0.1055	0.0204	4.68E-08	5.89E-05
Ssc.2152.1.A1_at	ASGA0102387	6	82,929,466	A	G	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	MARC0027805	6	83,038,514	A	G	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	ASGA0101913	6	83,194,382	A	G	-0.1094	0.0210	3.84E-08	5.28E-05
Ssc.2152.1.A1_at	MARC0045777	6	83,801,854	A	C	-0.1039	0.0206	9.81E-08	9.69E-05
Ssc.2152.1.A1_at	ALGA0035933	6	83,877,858	G	A	-0.1082	0.0212	7.83E-08	9.17E-05
Ssc.2152.1.A1_at	ASGA0084188	6	84,010,187	G	A	-0.1039	0.0206	9.81E-08	9.69E-05
Ssc.2152.1.A1_at	ASGA0101193	6	84,047,659	A	G	-0.1039	0.0206	9.81E-08	9.69E-05
Ssc.2152.1.A1_at	ALGA0116144	6	84,333,986	A	G	-0.1039	0.0206	9.81E-08	9.69E-05
Ssc.11158.2.S1_at	ALGA0035955	6	85,813,657	G	A	-0.2724	0.0504	6.73E-06	3.46E-02
Ssc.11158.2.S1_at	INRA0021679	6	85,830,735	C	A	-0.2871	0.0514	3.34E-06	2.06E-02
Ssc.11158.2.S1_at	ALGA0035975	6	86,899,252	A	G	-0.3251	0.0549	8.25E-07	8.48E-03
Ssc.11158.2.S1_at	ALGA0109191	6	89,504,250	A	G	-0.3333	0.0578	1.61E-06	1.24E-02
Ssc.2152.1.A1_at	MARC0015284	6	111,974,448	A	G	-0.0572	0.0146	3.76E-05	3.39E-02
Ssc.21969.1.A1_at	ASGA0090495	6	155,897,700	A	G	0.1383	0.0326	1.54E-06	4.44E-03
Ssc.21969.1.A1_at	ASGA0084445	6	155,970,768	G	A	-0.1319	0.0310	1.49E-06	4.44E-03
Ssc.21969.1.A1_at	MARC0017341	6	155,982,054	G	A	-0.1798	0.0302	1.49E-11	9.43E-08
Ssc.21969.1.A1_at	ASGA0089364	6	155,985,335	G	A	-0.1798	0.0302	1.49E-11	9.43E-08
Ssc.21969.1.A1_at	MARC0109147	6	156,179,469	A	G	0.1917	0.0308	1.91E-12	2.01E-08
Ssc.21969.1.A1_at	MARC0065489	6	156,197,571	A	G	0.1917	0.0308	1.91E-12	2.01E-08
Ssc.21969.1.A1_at	ALGA0119727	6	156,632,943	A	G	-0.1511	0.0346	7.37E-07	2.59E-03
Ssc.21969.1.A1_at	ASGA0084474	6	156,664,538	C	A	0.3204	0.0617	4.00E-09	1.80E-05
Ssc.21969.1.A1_at	M1GA0026172	6	157,003,423	G	A	0.1721	0.0338	7.99E-09	3.16E-05
Ssc.21969.1.A1_at	CASI0008589	6	157,126,627	A	G	0.1888	0.0303	1.67E-12	2.01E-08
Ssc.19634.1.S1_at	ASGA0032725	7	38,189,183	G	A	-0.2648	0.0604	1.86E-05	3.67E-02
Ssc.19634.1.S1_at	ASGA0032827	7	39,842,612	A	G	-0.2123	0.0483	1.77E-05	3.67E-02
Ssc.19634.1.S1_at	ALGA0040919	7	44,767,409	C	A	-0.2887	0.0544	2.13E-07	3.45E-03
Ssc.19634.1.S1_at	ASGA0033200	7	45,394,771	G	A	-0.2660	0.0501	2.18E-07	3.45E-03
Ssc.19634.1.S1_at	ALGA0040958	7	46,503,517	G	A	-0.2803	0.0613	8.02E-06	2.30E-02
Ssc.19634.1.S1_at	ALGA0040965	7	46,550,649	A	G	-0.2643	0.0568	5.54E-06	2.30E-02

Ssc.19634.1.S1_at	ALGA0041020	7	47,068,976	A	G	-0.2354	0.0461	6.28E-07	6.62E-03
Ssc.19634.1.S1_at	ALGA0041024	7	47,082,697	G	A	-0.2803	0.0613	8.02E-06	2.30E-02
Ssc.19634.1.S1_at	MARC0015432	7	47,106,377	G	A	-0.2803	0.0613	8.02E-06	2.30E-02
Ssc.19634.1.S1_at	ALGA0041077	7	48,105,743	A	C	-0.2316	0.0493	4.45E-06	2.30E-02
Ssc.19634.1.S1_at	ALGA0041087	7	48,179,859	G	A	-0.2692	0.0568	3.68E-06	2.30E-02
Ssc.19634.1.S1_at	ALGA0041328	7	50,750,507	G	A	-0.2803	0.0613	8.02E-06	2.30E-02
Ssc.19634.1.S1_at	ALGA0041332	7	50,781,071	G	A	-0.2803	0.0613	8.02E-06	2.30E-02
Ssc.15678.1.A1_s_at	MARC0037274	7	128,891,964	G	A	0.6206	0.1175	1.61E-08	5.08E-05
Ssc.21543.1.S1_at	MARC0037274	7	128,891,964	G	A	0.6834	0.1437	1.98E-06	6.94E-03
Ssc.15678.1.A1_s_at	MARC0017643	7	128,937,235	G	A	0.6206	0.1175	1.61E-08	5.08E-05
Ssc.21543.1.S1_at	MARC0017643	7	128,937,235	G	A	0.6834	0.1437	1.98E-06	6.94E-03
Ssc.15678.1.A1_s_at	ASGA0037072	7	128,969,247	G	A	0.6206	0.1175	1.61E-08	5.08E-05
Ssc.21543.1.S1_at	ASGA0037072	7	128,969,247	G	A	0.6834	0.1437	1.98E-06	6.94E-03
Ssc.15678.1.A1_s_at	ASGA0037093	7	129,119,274	G	A	0.6206	0.1175	1.61E-08	5.08E-05
Ssc.21543.1.S1_at	ASGA0037093	7	129,119,274	G	A	0.6834	0.1437	1.98E-06	6.94E-03
Ssc.15678.1.A1_s_at	ALGA0045692	7	129,184,305	A	T	0.6206	0.1175	1.61E-08	5.08E-05
Ssc.21543.1.S1_at	ALGA0045692	7	129,184,305	A	T	0.6834	0.1437	1.98E-06	6.94E-03
Ssc.15678.1.A1_s_at	ALGA0045709	7	129,269,847	A	G	0.6703	0.1144	3.70E-10	3.89E-06
Ssc.21543.1.S1_at	ALGA0045709	7	129,269,847	A	G	0.6286	0.1400	7.08E-06	2.23E-02
Ssc.15678.1.A1_s_at	ALGA0045737	7	129,340,461	A	C	0.6206	0.1175	1.61E-08	5.08E-05
Ssc.21543.1.S1_at	ALGA0045737	7	129,340,461	A	C	0.6834	0.1437	1.98E-06	6.94E-03
Ssc.15678.1.A1_s_at	BGIS0000745	7	129,389,307	G	A	0.5423	0.1305	8.85E-06	9.65E-03
Ssc.15678.1.A1_s_at	ALGA0045742	7	129,591,097	A	G	0.5423	0.1305	8.85E-06	9.65E-03
Ssc.15678.1.A1_s_at	ASGA0100209	7	129,604,415	A	G	0.6789	0.1168	5.03E-10	3.97E-06
Ssc.21543.1.S1_at	ASGA0100209	7	129,604,415	A	G	0.7284	0.1429	3.43E-07	3.59E-03
Ssc.15678.1.A1_s_at	ASGA0037172	7	129,819,024	G	A	0.5529	0.1307	6.05E-06	7.09E-03
Ssc.15678.1.A1_s_at	ASGA0037208	7	130,031,038	G	A	0.5529	0.1307	6.05E-06	7.09E-03
Ssc.15678.1.A1_s_at	ASGA0037216	7	130,099,514	A	G	0.5529	0.1307	6.05E-06	7.09E-03
Ssc.15678.1.A1_s_at	ALGA0045821	7	130,208,146	G	A	0.5529	0.1307	6.05E-06	7.09E-03
Ssc.15678.1.A1_s_at	ASGA0037233	7	130,353,771	G	C	0.5053	0.1039	2.00E-07	5.76E-04
Ssc.15678.1.A1_s_at	H3GA0023852	7	130,492,439	A	G	0.4874	0.1028	3.97E-07	8.36E-04
Ssc.15678.1.A1_s_at	ALGA0045833	7	130,512,758	A	G	0.4874	0.1028	3.97E-07	8.36E-04
Ssc.15678.1.A1_s_at	ASGA0037265	7	131,062,359	G	A	-0.5066	0.1120	1.30E-06	2.06E-03
Ssc.15678.1.A1_s_at	ASGA0037266	7	131,088,266	A	G	-0.5066	0.1120	1.30E-06	2.06E-03
Ssc.15678.1.A1_s_at	ASGA0037279	7	131,247,052	G	A	0.6767	0.1139	2.11E-10	3.33E-06
Ssc.21543.1.S1_at	ASGA0037279	7	131,247,052	G	A	0.7333	0.1394	1.43E-07	2.25E-03
Ssc.15678.1.A1_s_at	M1GA0011476	7	131,295,801	G	A	-0.5463	0.1160	4.77E-07	8.87E-04
Ssc.15678.1.A1_s_at	ASGA0037291	7	131,323,540	A	G	0.6767	0.1139	2.11E-10	3.33E-06
Ssc.21543.1.S1_at	ASGA0037291	7	131,323,540	A	G	0.7333	0.1394	1.43E-07	2.25E-03
Ssc.15678.1.A1_s_at	M1GA0011435	7	131,336,733	G	A	-0.4910	0.1076	1.05E-06	1.85E-03
Ssc.15678.1.A1_s_at	H3GA0023887	7	131,361,899	G	A	-0.5463	0.1160	4.77E-07	8.87E-04
Ssc.10298.1.A1_at	ASGA0098997	8	8,367,943	G	A	0.3931	0.0976	3.68E-05	3.43E-02
Ssc.10298.1.A1_at	ALGA0104225	8	8,934,560	G	A	0.4623	0.1122	2.41E-05	2.79E-02
Ssc.10298.1.A1_at	H3GA0024236	8	9,052,022	G	A	0.5250	0.0974	3.29E-08	7.42E-05
Ssc.10298.1.A1_at	ASGA0037695	8	9,080,127	C	A	0.5250	0.0974	3.29E-08	7.42E-05
Ssc.10298.1.A1_at	ALGA0046350	8	9,112,888	A	G	0.5250	0.0974	3.29E-08	7.42E-05
Ssc.10298.1.A1_at	ASGA0037704	8	9,152,556	G	A	0.4706	0.0909	1.14E-07	2.39E-04
Ssc.10298.1.A1_at	ALGA0046364	8	9,262,496	A	G	0.5250	0.0974	3.29E-08	7.42E-05
Ssc.10298.1.A1_at	MARC0054423	8	9,371,319	G	A	0.5413	0.0981	1.56E-08	4.94E-05
Ssc.10298.1.A1_at	ASGA0037733	8	9,480,475	A	G	0.5413	0.0981	1.56E-08	4.94E-05
Ssc.10298.1.A1_at	DRGA0008295	8	9,494,128	A	G	0.5413	0.0981	1.56E-08	4.94E-05
Ssc.10298.1.A1_at	MARC0036344	8	9,579,796	G	A	0.5413	0.0981	1.56E-08	4.94E-05
Ssc.10298.1.A1_at	DRGA0008300	8	9,800,284	A	G	0.3538	0.0775	2.93E-06	4.87E-03
Ssc.10298.1.A1_at	ALGA0121866	8	10,102,045	G	A	0.3335	0.0818	2.91E-05	2.79E-02
Ssc.10298.1.A1_at	ALGA0046395	8	10,116,124	G	A	0.3241	0.0794	2.87E-05	2.79E-02
Ssc.10298.1.A1_at	ALGA0112294	8	10,986,227	A	C	-0.4996	0.0835	8.67E-10	9.13E-06
Ssc.10298.1.A1_at	ALGA0111823	8	11,017,411	C	A	-0.3537	0.0816	9.01E-06	1.14E-02
Ssc.10298.1.A1_at	ASGA0096162	8	11,021,821	G	A	-0.5217	0.0836	1.57E-10	2.49E-06

Ssc.10298.1.A1_at	ASGA0097658	8	11,036,292	G	A	-0.3537	0.0816	9.01E-06	1.14E-02
Ssc.10298.1.A1_at	ALGA0122219	8	11,045,018	G	A	-0.3769	0.0816	2.24E-06	4.16E-03
Ssc.10298.1.A1_at	ASGA0037799	8	11,218,370	A	G	0.3438	0.0751	2.72E-06	4.78E-03
Ssc.10298.1.A1_at	M1GA0011802	8	11,285,754	G	A	0.5185	0.0883	1.75E-09	1.08E-05
Ssc.10298.1.A1_at	MARC0040190	8	11,319,179	G	A	0.3776	0.0794	1.09E-06	2.16E-03
Ssc.10298.1.A1_at	ASGA0092113	8	11,341,786	G	A	0.3313	0.0809	2.72E-05	2.79E-02
Ssc.10298.1.A1_at	H3GA0052944	8	11,343,093	G	A	0.3313	0.0809	2.72E-05	2.79E-02
Ssc.10298.1.A1_at	H3GA0024295	8	11,455,104	G	A	0.3330	0.0810	2.54E-05	2.79E-02
Ssc.10298.1.A1_at	MARC0101370	8	11,514,723	A	G	-0.3724	0.0829	4.21E-06	6.05E-03
Ssc.10298.1.A1_at	H3GA0024299	8	11,556,031	A	G	-0.3724	0.0829	4.21E-06	6.05E-03
Ssc.10298.1.A1_at	ASGA0037818	8	11,569,109	A	G	-0.3724	0.0829	4.21E-06	6.05E-03
Ssc.10298.1.A1_at	ALGA0046443	8	11,580,828	A	G	0.6211	0.0937	1.11E-11	3.50E-07
Ssc.10298.1.A1_at	ALGA0046479	8	11,946,016	A	C	0.4944	0.0845	2.04E-09	1.08E-05
Ssc.10298.1.A1_at	ASGA0037845	8	11,962,779	A	C	0.4944	0.0845	2.04E-09	1.08E-05
Ssc.7713.1.A1_at	ASGA0037859	8	12,476,033	G	A	0.2319	0.0510	1.95E-06	3.08E-02
Ssc.7713.1.A1_at	ALGA0106153	8	12,483,876	A	C	0.2319	0.0510	1.95E-06	3.08E-02
Ssc.29017.1.A1_at	ASGA0038548	8	33,235,728	A	G	0.2387	0.0527	2.27E-06	4.88E-02
Ssc.29017.1.A1_at	MARC0075877	8	33,365,922	G	A	0.2361	0.0530	3.31E-06	4.88E-02
Ssc.10298.1.A1_at	ASGA0103606	8	42,537,081	G	A	0.2953	0.0724	2.89E-05	2.79E-02
Ssc.19634.1.S1_at	ALGA0040508	9	104,532,905	A	G	-0.2648	0.0604	1.86E-05	3.67E-02
Ssc.7190.1.S1_at	ASGA0105497	9	118,021,130	G	A	0.1838	0.0448	2.14E-06	1.34E-02
Ssc.7190.1.S1_at	MARC0035219	9	118,070,358	A	C	0.1838	0.0448	2.14E-06	1.34E-02
Ssc.7190.1.S1_at	ASGA0100473	9	118,481,227	A	C	0.1704	0.0404	1.08E-06	1.13E-02
Ssc.7190.1.S1_at	ASGA0094122	9	118,508,396	A	G	0.1704	0.0404	1.08E-06	1.13E-02
Ssc.7190.1.S1_at	ASGA0044261	9	120,667,155	A	G	0.1923	0.0448	7.07E-07	1.13E-02
Ssc.9916.1.S1_at	ALGA0060373	11	3,914,900	G	A	0.0859	0.0182	4.14E-07	2.18E-03
Ssc.9916.1.S1_at	ALGA0060368	11	3,927,286	G	A	0.0859	0.0182	4.14E-07	2.18E-03
Ssc.9916.1.S1_at	ALGA0060433	11	4,308,845	A	G	0.0806	0.0177	1.05E-06	3.70E-03
Ssc.9916.1.S1_at	ALGA0060428	11	4,443,747	A	G	0.0806	0.0177	1.05E-06	3.70E-03
Ssc.9916.1.S1_at	ALGA0060825	11	11,542,003	C	A	-0.0768	0.0174	2.06E-06	6.50E-03
Ssc.9916.1.S1_at	ALGA0060833	11	11,590,497	G	A	-0.0660	0.0159	7.86E-06	1.38E-02
Ssc.9916.1.S1_at	H3GA0031333	11	11,880,203	G	A	-0.0714	0.0173	9.82E-06	1.55E-02
Ssc.9916.1.S1_at	H3GA0031354	11	12,175,996	G	A	-0.0850	0.0183	6.20E-07	2.80E-03
Ssc.9916.1.S1_at	ASGA0049857	11	12,339,098	C	A	-0.0807	0.0154	1.89E-08	1.99E-04
Ssc.9916.1.S1_at	H3GA0031355	11	12,363,638	G	A	-0.0807	0.0154	1.89E-08	1.99E-04
Ssc.9916.1.S1_at	DRGA0010855	11	12,687,697	A	G	-0.0667	0.0165	1.35E-05	1.93E-02
Ssc.9916.1.S1_at	ASGA0049908	11	13,967,580	A	G	-0.0692	0.0167	8.79E-06	1.46E-02
Ssc.9916.1.S1_at	ASGA0049912	11	14,028,142	A	C	0.0694	0.0162	4.15E-06	1.09E-02
Ssc.9916.1.S1_at	MARC0084745	11	14,044,193	A	C	-0.0836	0.0171	1.39E-07	1.10E-03
Ssc.9916.1.S1_at	MARC0074353	11	15,364,802	A	G	-0.0690	0.0170	1.24E-05	1.87E-02
Ssc.9916.1.S1_at	MARC0031054	11	15,537,140	G	A	-0.0761	0.0183	7.60E-06	1.38E-02
Ssc.9916.1.S1_at	MARC0028819	11	16,996,424	G	A	-0.0668	0.0157	4.71E-06	1.14E-02
Ssc.9916.1.S1_at	ALGA0061159	11	18,229,231	G	A	-0.0680	0.0178	3.90E-05	4.26E-02
Ssc.9916.1.S1_at	ALGA0110767	11	19,969,481	G	A	-0.0664	0.0157	5.38E-06	1.21E-02
Ssc.9916.1.S1_at	ASGA0083665	11	19,977,848	A	G	-0.0738	0.0175	5.76E-06	1.21E-02
Ssc.9916.1.S1_at	DRGA0010927	11	20,085,816	A	G	-0.1335	0.0311	3.98E-06	1.09E-02
Ssc.9916.1.S1_at	ALGA0106317	11	20,902,453	A	G	-0.0691	0.0165	7.00E-06	1.38E-02
Ssc.9916.1.S1_at	MARC0019798	11	23,315,076	A	G	-0.0668	0.0170	2.39E-05	3.15E-02
Ssc.9916.1.S1_at	DRGA0010960	11	23,463,154	G	A	-0.1167	0.0303	3.63E-05	4.14E-02
Ssc.6613.1.A1_at	H3GA0032794	11	84,687,502	A	G	0.1481	0.0295	1.26E-06	4.00E-02
Ssc.10589.1.A1_at	DRGA0012154	13	19,683,797	C	A	-0.1944	0.0360	2.97E-05	1.12E-02
Ssc.10589.1.A1_at	MARC0098145	13	19,750,520	C	A	-0.1802	0.0341	4.40E-05	1.52E-02
Ssc.24608.1.S1_at	DIAS0002174	13	19,774,122	G	A	0.1019	0.0227	2.97E-06	4.69E-02
Ssc.24608.1.S1_at	ALGA0122535	13	19,859,830	G	A	0.1074	0.0233	1.57E-06	4.69E-02
Ssc.10589.1.A1_at	MARC0060442	13	25,730,204	A	G	0.1676	0.0337	1.23E-04	3.24E-02
Ssc.24997.1.S1_at	ALGA0124300	13	26,286,725	C	A	0.0754	0.0191	6.61E-06	2.26E-02
Ssc.24997.1.S1_at	ASGA0056722	13	26,522,668	A	G	0.0754	0.0191	6.61E-06	2.26E-02
Ssc.24997.1.S1_at	MARC0063025	13	26,594,270	G	A	0.0754	0.0191	6.61E-06	2.26E-02

Ssc.10589.1.A1_at	ALGA0068966	13	26,794,660	C	A	0.1813	0.0329	2.02E-05	8.43E-03
Ssc.24997.1.S1_at	ALGA0068973	13	26,845,333	A	G	0.0819	0.0199	2.45E-06	1.25E-02
Ssc.24997.1.S1_at	DRGA0012225	13	27,302,274	G	A	0.0819	0.0199	2.45E-06	1.25E-02
Ssc.24997.1.S1_at	MARC0003563	13	27,793,745	A	C	0.0819	0.0199	2.45E-06	1.25E-02
Ssc.24997.1.S1_at	ALGA0069067	13	27,837,250	G	A	0.0819	0.0199	2.45E-06	1.25E-02
Ssc.24997.1.S1_at	H3GA0035928	13	27,911,595	A	G	0.0819	0.0199	2.45E-06	1.25E-02
Ssc.10589.1.A1_at	ALGA0069124	13	28,893,435	A	G	-0.2101	0.0406	6.31E-05	2.00E-02
Ssc.10589.1.A1_at	MARC0068947	13	30,329,083	G	A	0.1837	0.0329	1.59E-05	7.19E-03
Ssc.10589.1.A1_at	ASGA0057018	13	31,650,967	A	G	0.1866	0.0334	1.54E-05	7.05E-03
Ssc.10589.1.A1_at	H3GA0036121	13	34,526,276	G	A	-0.2182	0.0396	2.05E-05	8.43E-03
Ssc.10589.1.A1_at	MARC0052461	13	34,606,587	A	G	-0.2041	0.0370	2.03E-05	8.43E-03
Ssc.10589.1.A1_at	ASGA0099106	13	35,628,206	G	A	-0.1769	0.0350	9.45E-05	2.69E-02
Ssc.10589.1.A1_at	ALGA0069510	13	36,886,746	A	G	-0.1772	0.0340	5.65E-05	1.80E-02
Ssc.10589.1.A1_at	MARC0064610	13	36,921,951	A	G	-0.1772	0.0340	5.65E-05	1.80E-02
Ssc.10589.1.A1_at	H3GA0036210	13	36,964,009	G	A	-0.1772	0.0340	5.65E-05	1.80E-02
Ssc.10589.1.A1_at	ALGA0069839	13	46,063,345	G	A	-0.2452	0.0403	2.51E-06	4.18E-03
Ssc.10589.1.A1_at	DRGA0012567	13	50,853,035	G	A	-0.2042	0.0371	2.13E-05	8.51E-03
Ssc.10589.1.A1_at	ASGA0057479	13	51,612,757	G	A	-0.1557	0.0315	1.32E-04	3.35E-02
Ssc.10589.1.A1_at	ALGA0069967	13	51,650,319	A	G	-0.1557	0.0315	1.32E-04	3.35E-02
Ssc.10589.1.A1_at	ALGA0069988	13	52,016,944	G	A	-0.1557	0.0315	1.32E-04	3.35E-02
Ssc.10589.1.A1_at	MARC0006686	13	52,640,638	A	G	-0.1990	0.0359	1.78E-05	7.70E-03
Ssc.10589.1.A1_at	H3GA0036416	13	52,715,736	A	G	-0.1990	0.0371	3.40E-05	1.22E-02
Ssc.10589.1.A1_at	H3GA0036418	13	52,729,074	A	G	-0.1990	0.0371	3.40E-05	1.22E-02
Ssc.10589.1.A1_at	ALGA0115114	13	53,528,819	A	C	-0.1960	0.0363	2.96E-05	1.12E-02
Ssc.10589.1.A1_at	ASGA0084088	13	53,675,522	G	A	-0.1960	0.0363	2.96E-05	1.12E-02
Ssc.10589.1.A1_at	ALGA0117603	13	53,877,058	A	G	-0.1960	0.0363	2.96E-05	1.12E-02
Ssc.10589.1.A1_at	H3GA0036546	13	59,740,715	G	A	-0.2024	0.0362	1.53E-05	7.05E-03
Ssc.10589.1.A1_at	ASGA0057720	13	59,841,342	G	A	-0.2024	0.0362	1.53E-05	7.05E-03
Ssc.10589.1.A1_at	ALGA0070362	13	60,439,469	G	A	-0.2052	0.0365	1.37E-05	6.58E-03
Ssc.10589.1.A1_at	H3GA0036571	13	60,609,657	A	G	-0.2320	0.0357	5.05E-07	2.28E-03
Ssc.10589.1.A1_at	ALGA0070387	13	60,801,744	G	A	0.2082	0.0367	1.18E-05	6.20E-03
Ssc.10589.1.A1_at	ALGA0070388	13	60,807,418	G	A	0.2082	0.0367	1.18E-05	6.20E-03
Ssc.10589.1.A1_at	ALGA0070393	13	60,881,758	A	G	0.2082	0.0367	1.18E-05	6.20E-03
Ssc.10589.1.A1_at	ALGA0070390	13	61,014,675	G	A	0.2082	0.0367	1.18E-05	6.20E-03
Ssc.10589.1.A1_at	H3GA0036584	13	61,069,055	A	C	-0.2052	0.0365	1.37E-05	6.58E-03
Ssc.10589.1.A1_at	ASGA0057781	13	61,157,938	G	A	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	ALGA0070395	13	61,211,799	G	A	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	DRGA0012455	13	61,231,210	A	C	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	ASGA0057778	13	61,245,821	A	G	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	H3GA0036585	13	61,251,568	G	A	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	SIRI0000331	13	61,327,131	A	G	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	H3GA0053132	13	61,452,089	G	A	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	MARC0067064	13	61,565,018	C	A	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	ASGA0099219	13	61,627,828	A	G	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	ALGA0070419	13	61,964,562	A	G	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	MARC0020998	13	62,080,025	G	A	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	ASGA0094034	13	62,566,678	A	G	0.2082	0.0367	1.18E-05	6.20E-03
Ssc.10589.1.A1_at	H3GA0036594	13	62,640,550	G	A	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	ASGA0057799	13	63,570,934	A	C	-0.2139	0.0338	9.88E-07	2.84E-03
Ssc.10589.1.A1_at	MARC0034409	13	63,784,154	A	T	-0.2139	0.0338	9.88E-07	2.84E-03
Ssc.10589.1.A1_at	ALGA0070448	13	63,937,862	C	A	-0.2139	0.0338	9.88E-07	2.84E-03
Ssc.10589.1.A1_at	ASGA0057807	13	63,946,644	A	G	-0.2139	0.0338	9.88E-07	2.84E-03
Ssc.10589.1.A1_at	ALGA0070458	13	63,994,105	G	A	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	ASGA0057808	13	64,033,973	C	A	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	DRGA0012479	13	64,493,086	G	A	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	ALGA0070493	13	64,933,643	G	A	-0.2320	0.0357	5.05E-07	2.28E-03
Ssc.10589.1.A1_at	DRGA0012487	13	64,999,793	A	G	0.2082	0.0367	1.18E-05	6.20E-03
Ssc.10589.1.A1_at	ASGA0057818	13	65,025,003	A	G	0.2001	0.0354	1.26E-05	6.20E-03

Ssc.10589.1.A1_at	ASGA0057820	13	65,041,227	G	A	0.2082	0.0367	1.18E-05	6.20E-03
Ssc.10589.1.A1_at	ALGA0070500	13	65,054,631	A	G	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	ASGA0091483	13	65,209,239	A	G	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	ASGA0105748	13	65,211,841	A	G	0.2001	0.0354	1.26E-05	6.20E-03
Ssc.10589.1.A1_at	H3GA0036622	13	65,952,730	A	G	-0.2030	0.0359	1.23E-05	6.20E-03
Ssc.10589.1.A1_at	MARC0096215	13	66,268,000	G	A	-0.2072	0.0355	6.35E-06	6.20E-03
Ssc.10589.1.A1_at	MARC0077830	13	66,315,906	G	A	-0.2072	0.0355	6.35E-06	6.20E-03
Ssc.10589.1.A1_at	ALGA0070545	13	66,348,482	A	C	-0.2072	0.0355	6.35E-06	6.20E-03
Ssc.10589.1.A1_at	ALGA0070557	13	66,460,974	G	A	0.2133	0.0362	5.23E-06	6.20E-03
Ssc.10589.1.A1_at	MARC0110081	13	66,717,995	A	C	-0.2072	0.0355	6.35E-06	6.20E-03
Ssc.10589.1.A1_at	ALGA0070577	13	67,677,342	G	A	-0.2355	0.0373	1.08E-06	2.84E-03
Ssc.10589.1.A1_at	ALGA0070612	13	68,279,611	G	A	0.1873	0.0348	3.16E-05	1.17E-02
Ssc.10589.1.A1_at	DRGA0012520	13	68,402,608	A	G	-0.2390	0.0361	3.13E-07	1.98E-03
Ssc.10589.1.A1_at	ALGA0070619	13	68,529,027	A	G	0.1951	0.0361	2.97E-05	1.12E-02
Ssc.10589.1.A1_at	ALGA0109826	13	68,552,642	G	A	-0.1865	0.0353	4.48E-05	1.52E-02
Ssc.10589.1.A1_at	DRGA0012530	13	69,671,505	G	A	-0.1865	0.0353	4.48E-05	1.52E-02
Ssc.10589.1.A1_at	MARC0080856	13	70,349,883	A	G	-0.1865	0.0353	4.48E-05	1.52E-02
Ssc.10589.1.A1_at	ALGA0070696	13	71,946,567	A	G	0.2117	0.0430	1.39E-04	3.43E-02
Ssc.10589.1.A1_at	MARC0052770	13	72,033,973	A	G	-0.2042	0.0371	2.13E-05	8.51E-03
Ssc.10589.1.A1_at	MARC0015967	13	72,517,319	A	G	-0.1772	0.0337	4.79E-05	1.58E-02
Ssc.10589.1.A1_at	ALGA0115717	13	72,572,816	G	A	-0.1772	0.0337	4.79E-05	1.58E-02
Ssc.10589.1.A1_at	ALGA0070724	13	73,082,286	A	G	0.1995	0.0403	1.31E-04	3.35E-02
Ssc.10589.1.A1_at	H3GA0036708	13	73,241,763	G	A	-0.1650	0.0325	8.53E-05	2.54E-02
Ssc.10589.1.A1_at	H3GA0036721	13	73,787,637	G	A	0.1740	0.0347	1.07E-04	2.94E-02
Ssc.10589.1.A1_at	ASGA0085037	13	78,219,919	G	A	-0.2133	0.0343	1.47E-06	3.32E-03
Ssc.10589.1.A1_at	ASGA0085655	13	78,397,547	A	G	0.1740	0.0347	1.07E-04	2.94E-02
Ssc.10589.1.A1_at	ALGA0117923	13	80,236,129	G	A	0.2061	0.0364	1.18E-05	6.20E-03
Ssc.10589.1.A1_at	M1GA0025421	13	80,585,007	A	G	-0.1721	0.0339	8.84E-05	2.54E-02
Ssc.10589.1.A1_at	ALGA0070890	13	80,968,195	G	A	0.1796	0.0351	7.62E-05	2.32E-02
Ssc.10589.1.A1_at	H3GA0036832	13	80,987,722	G	A	0.1796	0.0351	7.62E-05	2.32E-02
Ssc.10589.1.A1_at	H3GA0036836	13	81,015,331	A	G	-0.2579	0.0366	5.26E-08	5.54E-04
Ssc.10589.1.A1_at	ALGA0070894	13	81,059,426	A	G	-0.2579	0.0366	5.26E-08	5.54E-04
Ssc.10589.1.A1_at	ALGA0070897	13	81,083,948	G	A	0.2098	0.0368	1.07E-05	6.20E-03
Ssc.10589.1.A1_at	ALGA0070902	13	81,210,429	A	C	-0.1646	0.0320	7.09E-05	2.20E-02
Ssc.10589.1.A1_at	MARC0002975	13	81,236,265	A	C	-0.1721	0.0339	8.84E-05	2.54E-02
Ssc.10589.1.A1_at	MARC0070868	13	81,253,205	A	G	-0.1721	0.0339	8.84E-05	2.54E-02
Ssc.10589.1.A1_at	ASGA0058210	13	83,310,705	G	A	-0.1962	0.0355	1.92E-05	8.20E-03
Ssc.10589.1.A1_at	ALGA0071026	13	84,175,943	C	A	-0.2017	0.0379	3.93E-05	1.40E-02
Ssc.10589.1.A1_at	ASGA0058239	13	84,277,803	A	G	-0.1751	0.0358	1.55E-04	3.78E-02
Ssc.10589.1.A1_at	MARC0073115	13	87,702,788	G	A	0.2085	0.0359	7.09E-06	6.20E-03
Ssc.10589.1.A1_at	ALGA0071141	13	88,045,385	G	A	-0.2279	0.0399	9.85E-06	6.20E-03
Ssc.10589.1.A1_at	H3GA0036968	13	88,076,611	A	G	0.2051	0.0356	8.57E-06	6.20E-03
Ssc.10589.1.A1_at	DRGA0012624	13	88,102,755	A	G	-0.2401	0.0357	1.99E-07	1.58E-03
Ssc.10589.1.A1_at	ALGA0071165	13	88,513,779	G	A	0.2266	0.0369	2.06E-06	3.69E-03
Ssc.10589.1.A1_at	MARC0009028	13	88,701,711	G	A	0.1937	0.0387	1.09E-04	2.94E-02
Ssc.10589.1.A1_at	ALGA0071259	13	89,076,273	C	A	0.1937	0.0387	1.09E-04	2.94E-02
Ssc.10589.1.A1_at	H3GA0037017	13	89,177,420	C	A	-0.2616	0.0371	5.18E-08	5.54E-04
Ssc.10589.1.A1_at	ASGA0058397	13	89,417,869	A	G	0.2107	0.0343	2.05E-06	3.69E-03
Ssc.10589.1.A1_at	H3GA0037030	13	89,919,771	A	G	-0.1858	0.0364	8.02E-05	2.41E-02
Ssc.10589.1.A1_at	ASGA0088975	13	93,191,917	A	G	-0.2211	0.0370	3.90E-06	5.36E-03
Ssc.10589.1.A1_at	ASGA0058540	13	98,646,754	A	G	0.1920	0.0311	1.87E-06	3.69E-03
Ssc.10589.1.A1_at	MARC0066829	13	99,515,364	G	A	-0.2165	0.0353	2.10E-06	3.69E-03
Ssc.10589.1.A1_at	ASGA0058567	13	99,849,670	A	C	0.1888	0.0339	1.63E-05	7.25E-03
Ssc.10589.1.A1_at	MARC0097146	13	101,533,414	A	C	0.1741	0.0313	1.68E-05	7.39E-03
Ssc.10589.1.A1_at	ALGA0105535	13	102,458,997	A	C	-0.1807	0.0336	3.26E-05	1.20E-02
Ssc.10589.1.A1_at	MARC0073759	13	103,194,490	G	A	-0.1882	0.0321	5.97E-06	6.20E-03
Ssc.10589.1.A1_at	ASGA0058623	13	107,213,278	A	G	0.1884	0.0378	1.18E-04	3.13E-02
Ssc.10589.1.A1_at	MARC0046213	13	117,497,966	G	A	-0.1890	0.0328	8.36E-06	6.20E-03

Ssc.10589.1.A1_at	MARC0056838	13	120,482,840	A	G	-0.1548	0.0309	1.05E-04	2.92E-02
Ssc.10589.1.A1_at	INRA0040886	13	124,746,808	A	G	0.1724	0.0349	1.36E-04	3.39E-02
Ssc.10589.1.A1_at	MARC0066980	13	124,921,806	G	A	0.1724	0.0349	1.36E-04	3.39E-02
Ssc.10589.1.A1_at	ALGA0071780	13	124,983,364	A	G	0.1724	0.0349	1.36E-04	3.39E-02
Ssc.10589.1.A1_at	ALGA0071791	13	125,729,926	G	A	-0.1990	0.0399	1.13E-04	3.03E-02
Ssc.10589.1.A1_at	MARC0057672	13	126,830,429	A	G	0.1691	0.0352	2.00E-04	4.68E-02
Ssc.24542.1.S1_at	INRA0040972	13	134,933,329	A	C	-0.4231	0.0937	1.43E-05	4.91E-02
Ssc.24542.1.S1_at	MARC0021665	13	135,177,274	G	A	-0.3609	0.0823	2.53E-05	4.96E-02
Ssc.24542.1.S1_at	MARC0112508	13	135,180,016	G	A	-0.3609	0.0823	2.53E-05	4.96E-02
Ssc.24542.1.S1_at	MARC0052863	13	135,294,742	G	A	-0.3609	0.0823	2.53E-05	4.96E-02
Ssc.24542.1.S1_at	ASGA0058792	13	135,911,518	A	G	-0.4156	0.0936	2.02E-05	4.91E-02
Ssc.24542.1.S1_at	ALGA0071929	13	136,438,657	G	A	-0.4179	0.0940	1.94E-05	4.91E-02
Ssc.24542.1.S1_at	INRA0040979	13	136,460,931	G	A	-0.4179	0.0940	1.94E-05	4.91E-02
Ssc.24542.1.S1_at	ASGA0058796	13	136,602,514	A	G	-0.4179	0.0940	1.94E-05	4.91E-02
Ssc.24542.1.S1_at	ASGA0058797	13	136,647,355	A	G	-0.4179	0.0940	1.94E-05	4.91E-02
Ssc.24542.1.S1_at	MARC0061118	13	136,709,417	A	G	-0.4179	0.0940	1.94E-05	4.91E-02
Ssc.24542.1.S1_at	MARC0045530	13	136,902,376	G	A	-0.4211	0.0936	1.56E-05	4.91E-02
Ssc.24542.1.S1_at	H3GA0055799	13	137,254,030	G	A	-0.4211	0.0936	1.56E-05	4.91E-02
Ssc.24542.1.S1_at	ASGA0058810	13	138,039,183	A	G	-0.4211	0.0936	1.56E-05	4.91E-02
Ssc.24542.1.S1_at	DRGA0012872	13	138,591,603	A	G	0.3596	0.0825	2.83E-05	4.96E-02
Ssc.24542.1.S1_at	MARC0013057	13	138,710,512	A	G	-0.4211	0.0936	1.56E-05	4.91E-02
Ssc.24542.1.S1_at	ASGA0058820	13	138,941,535	A	C	-0.4211	0.0936	1.56E-05	4.91E-02
Ssc.24542.1.S1_at	H3GA0056086	13	140,150,111	A	C	-0.3380	0.0773	2.68E-05	4.96E-02
Ssc.24542.1.S1_at	ASGA0104081	13	140,308,510	C	A	-0.3928	0.0885	2.02E-05	4.91E-02
Ssc.10589.1.A1_at	ALGA0072303	13	154,043,749	C	A	-0.2088	0.0428	1.64E-04	3.92E-02
Ssc.10589.1.A1_at	ALGA0072308	13	154,103,781	G	A	-0.2088	0.0428	1.64E-04	3.92E-02
Ssc.21861.1.S1_at	MARC0094668	14	31,671,713	G	A	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	DIAS0000600	14	31,695,899	A	G	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ASGA0062507	14	31,797,751	G	A	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	MARC0035603	14	31,924,011	G	A	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	INRA0043413	14	31,940,974	C	A	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ALGA0076686	14	31,983,579	G	A	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	MARC0010192	14	32,000,259	A	G	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	MARC0051173	14	32,082,231	A	G	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ASGA0062523	14	32,537,144	G	A	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ASGA0062526	14	32,650,321	G	A	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ASGA0062531	14	32,881,675	A	G	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ASGA0062546	14	33,315,496	A	G	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ALGA0076722	14	33,341,083	A	C	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ALGA0076725	14	33,365,481	A	G	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	INRA0043453	14	33,420,925	A	G	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ASGA0062551	14	33,523,556	G	A	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ALGA0076734	14	33,775,751	A	G	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	DIAS0001236	14	33,833,169	G	A	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ALGA0076738	14	33,939,592	A	G	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ASGA0062561	14	33,959,799	C	A	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	H3GA0039681	14	34,089,605	G	A	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ASGA0062577	14	34,321,513	G	A	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ASGA0062588	14	34,461,240	A	G	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	ASGA0062593	14	34,512,344	A	G	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	INRA0043496	14	34,666,553	A	C	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	H3GA0039700	14	34,930,114	A	G	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.21861.1.S1_at	MARC0078186	14	34,956,919	A	C	0.4171	0.0992	1.64E-05	1.72E-02
Ssc.9109.1.A1_at	MARC0087847	14	100,004,352	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065349	14	100,071,766	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065354	14	100,097,831	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065360	14	100,133,766	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065361	14	100,162,325	C	A	-0.0198	0.0050	8.72E-05	1.36E-02

Ssc.9109.1.A1_at	ALGA0079968	14	100,212,153	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065389	14	100,376,668	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	SIRI0000848	14	100,494,083	A	G	0.0219	0.0048	5.49E-06	1.26E-02
Ssc.9109.1.A1_at	INRA0045783	14	100,494,371	A	G	0.0219	0.0048	5.49E-06	1.26E-02
Ssc.9109.1.A1_at	MARC0090892	14	100,862,271	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0011234	14	100,938,586	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0093262	14	100,971,708	C	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014281	14	101,124,119	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	CASI0009035	14	101,145,595	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014282	14	101,161,138	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041573	14	101,181,073	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	INRA0045792	14	101,193,342	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080004	14	101,214,975	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041574	14	101,227,861	A	C	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065412	14	101,350,552	G	A	-0.0196	0.0052	1.82E-04	2.61E-02
Ssc.9109.1.A1_at	H3GA0041580	14	101,532,615	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014290	14	101,566,065	A	G	0.0193	0.0050	1.31E-04	1.96E-02
Ssc.9109.1.A1_at	ALGA0080034	14	101,983,348	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	INRA0045816	14	101,995,631	A	C	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080038	14	102,136,878	A	C	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	SIRI0000335	14	102,209,608	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0101253	14	102,244,897	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080040	14	102,310,696	C	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	INRA0045833	14	102,329,888	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	INRA0045843	14	102,798,434	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014304	14	102,947,543	C	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014307	14	102,988,071	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065430	14	103,045,309	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0104305	14	103,168,211	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065434	14	103,312,088	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065436	14	103,343,664	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080094	14	103,900,532	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	INRA0045898	14	103,915,659	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0070934	14	103,936,269	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065448	14	104,029,253	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080103	14	104,083,021	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014325	14	104,117,355	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014327	14	104,153,804	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080105	14	104,179,804	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014328	14	104,195,215	A	C	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080107	14	104,231,224	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014332	14	104,267,059	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014334	14	104,333,617	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014336	14	104,355,465	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065461	14	104,441,890	A	C	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	INRA0045934	14	104,459,994	A	C	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065469	14	104,505,916	A	C	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080129	14	104,552,293	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080132	14	104,591,948	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041604	14	104,637,815	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080136	14	104,661,126	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065470	14	104,699,566	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041605	14	104,712,773	G	A	0.0215	0.0047	6.70E-06	1.26E-02
Ssc.9109.1.A1_at	INRA0045948	14	104,759,248	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080141	14	104,872,741	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065472	14	104,885,629	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014352	14	105,084,009	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0017943	14	105,375,735	A	G	-0.0198	0.0050	8.72E-05	1.36E-02

Ssc.9109.1.A1_at	MARC0086582	14	105,383,689	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DIAS0004751	14	105,635,953	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080176	14	105,701,530	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	INRA0045966	14	105,723,423	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0008683	14	105,743,632	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080177	14	105,760,321	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080189	14	105,847,248	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014372	14	106,074,877	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	INRA0045986	14	106,118,402	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014376	14	106,446,655	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080212	14	106,552,245	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080216	14	106,614,714	C	A	-0.0194	0.0050	1.46E-04	2.15E-02
Ssc.9109.1.A1_at	ASGA0065507	14	106,653,236	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041638	14	106,860,689	A	C	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065520	14	107,057,346	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0051749	14	107,210,903	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080246	14	107,218,916	G	A	-0.0198	0.0050	8.41E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065523	14	107,245,669	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065524	14	107,361,548	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080248	14	107,385,384	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041643	14	107,407,571	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041644	14	107,457,286	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0096934	14	107,513,181	A	G	0.0215	0.0047	6.70E-06	1.26E-02
Ssc.9109.1.A1_at	ALGA0080256	14	107,582,928	A	C	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0001013	14	107,602,780	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080263	14	107,613,604	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065533	14	107,731,068	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0069003	14	107,770,051	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0038193	14	107,783,569	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065540	14	107,827,025	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0043296	14	107,893,125	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065545	14	107,922,056	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065547	14	108,016,508	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014408	14	108,070,980	G	A	-0.0195	0.0051	1.34E-04	1.99E-02
Ssc.9109.1.A1_at	ALGA0080326	14	108,168,784	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041669	14	108,220,063	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080335	14	108,264,936	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080343	14	108,386,204	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065571	14	108,411,849	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080340	14	108,453,123	A	C	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041684	14	108,719,339	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	UMB10000047	14	108,788,173	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	INRA0046109	14	108,892,579	C	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080383	14	109,190,861	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080384	14	109,194,423	C	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0058294	14	109,285,369	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065594	14	109,526,894	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041699	14	109,557,530	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065598	14	109,659,225	G	A	0.0215	0.0047	6.70E-06	1.26E-02
Ssc.9109.1.A1_at	ALGA0080413	14	109,761,857	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080414	14	109,782,472	G	C	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065613	14	109,802,875	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080416	14	109,827,501	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065623	14	109,952,277	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065624	14	109,965,497	A	G	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080430	14	109,988,613	G	A	-0.0198	0.0050	8.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065636	14	110,165,978	G	A	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	M1GA0018990	14	110,187,467	G	A	-0.0203	0.0050	5.49E-05	1.36E-02

Ssc.9109.1.A1_at	H3GA0041722	14	110,272,404	G	A	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065640	14	110,311,908	A	G	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	INRA0046264	14	110,453,567	G	A	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065655	14	110,467,350	A	G	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080480	14	110,599,338	G	A	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0028529	14	110,624,980	G	A	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041735	14	110,638,187	G	A	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080500	14	110,983,342	A	C	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041738	14	111,089,602	A	G	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080501	14	111,127,741	C	A	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0028812	14	111,131,649	G	A	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065681	14	111,211,003	A	G	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080507	14	111,247,792	G	A	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065685	14	111,261,959	A	G	-0.0203	0.0050	5.49E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080524	14	111,426,258	C	A	0.0213	0.0050	2.78E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041753	14	111,454,442	A	G	-0.0219	0.0049	8.77E-06	1.26E-02
Ssc.9109.1.A1_at	MARC0016212	14	111,786,019	A	G	-0.0226	0.0048	3.30E-06	1.26E-02
Ssc.9109.1.A1_at	ASGA0065732	14	111,866,876	A	G	-0.0192	0.0048	7.86E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080585	14	112,345,300	G	A	-0.0193	0.0048	7.90E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014434	14	112,732,635	A	C	-0.0190	0.0047	5.92E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014437	14	112,992,396	C	A	-0.0190	0.0047	5.92E-05	1.36E-02
Ssc.9109.1.A1_at	DRGA0014438	14	113,074,497	G	A	-0.0190	0.0047	5.92E-05	1.36E-02
Ssc.9109.1.A1_at	MARC0071608	14	113,308,461	G	A	-0.0190	0.0047	5.92E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065778	14	113,333,663	A	G	0.0193	0.0043	9.97E-06	1.26E-02
Ssc.9109.1.A1_at	ASGA0065780	14	113,376,632	G	A	-0.0203	0.0047	2.14E-05	1.34E-02
Ssc.9109.1.A1_at	ASGA0065790	14	113,554,994	G	A	-0.0203	0.0047	2.14E-05	1.34E-02
Ssc.9109.1.A1_at	ALGA0080674	14	113,973,779	C	A	0.0237	0.0047	4.90E-07	7.74E-03
Ssc.9109.1.A1_at	BGIS0007629	14	114,007,859	A	G	0.0237	0.0047	4.90E-07	7.74E-03
Ssc.9109.1.A1_at	ALGA0080681	14	114,148,956	G	A	-0.0203	0.0047	2.14E-05	1.34E-02
Ssc.9109.1.A1_at	MARC0025599	14	114,194,502	A	G	-0.0203	0.0047	2.14E-05	1.34E-02
Ssc.9109.1.A1_at	ALGA0112524	14	114,586,489	G	A	-0.0204	0.0048	2.20E-05	1.34E-02
Ssc.9109.1.A1_at	ASGA0065819	14	114,587,543	G	A	-0.0204	0.0048	2.20E-05	1.34E-02
Ssc.9109.1.A1_at	ALGA0080697	14	114,610,574	A	G	-0.0204	0.0048	2.20E-05	1.34E-02
Ssc.9109.1.A1_at	MARC0086765	14	114,735,306	A	G	0.0203	0.0044	4.05E-06	1.26E-02
Ssc.9109.1.A1_at	ASGA0065826	14	114,803,353	A	G	0.0203	0.0044	4.05E-06	1.26E-02
Ssc.9109.1.A1_at	MARC0055120	14	114,808,000	A	G	0.0203	0.0044	4.05E-06	1.26E-02
Ssc.9109.1.A1_at	MARC0027573	14	115,043,532	A	G	0.0188	0.0043	1.71E-05	1.26E-02
Ssc.9109.1.A1_at	ASGA0065838	14	115,122,766	G	A	0.0188	0.0043	1.71E-05	1.26E-02
Ssc.9109.1.A1_at	H3GA0041837	14	115,190,272	A	G	0.0188	0.0043	1.71E-05	1.26E-02
Ssc.9109.1.A1_at	H3GA0041842	14	115,222,301	G	A	0.0188	0.0043	1.71E-05	1.26E-02
Ssc.9109.1.A1_at	ASGA0092332	14	115,241,948	G	A	0.0188	0.0043	1.71E-05	1.26E-02
Ssc.9109.1.A1_at	ALGA0080725	14	115,284,002	G	A	0.0188	0.0043	1.71E-05	1.26E-02
Ssc.9109.1.A1_at	ALGA0080726	14	115,295,550	G	C	0.0188	0.0043	1.71E-05	1.26E-02
Ssc.9109.1.A1_at	ASGA0065840	14	115,447,844	A	G	0.0188	0.0043	1.71E-05	1.26E-02
Ssc.9109.1.A1_at	ALGA0080732	14	115,729,764	A	G	0.0188	0.0043	1.71E-05	1.26E-02
Ssc.9109.1.A1_at	INRA0046441	14	115,742,329	A	G	0.0181	0.0043	3.72E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065848	14	115,834,469	A	G	0.0188	0.0043	1.71E-05	1.26E-02
Ssc.9109.1.A1_at	ALGA0117921	14	115,960,354	A	G	0.0188	0.0043	1.71E-05	1.26E-02
Ssc.9109.1.A1_at	ALGA0080773	14	116,331,877	A	G	0.0188	0.0043	1.88E-05	1.32E-02
Ssc.9109.1.A1_at	ALGA0080776	14	116,383,375	G	A	0.0188	0.0043	1.71E-05	1.26E-02
Ssc.9109.1.A1_at	H3GA0041864	14	116,406,028	G	A	0.0181	0.0043	3.72E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080788	14	116,520,823	G	A	0.0186	0.0043	1.62E-05	1.26E-02
Ssc.9109.1.A1_at	MARC0040955	14	116,538,288	G	A	0.0188	0.0043	1.71E-05	1.26E-02
Ssc.9109.1.A1_at	MARC0040736	14	116,560,598	G	A	0.0199	0.0050	7.53E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080802	14	116,602,344	G	A	-0.0182	0.0043	3.02E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041895	14	116,650,528	G	A	0.0182	0.0043	3.02E-05	1.36E-02
Ssc.9109.1.A1_at	H3GA0041897	14	116,664,345	C	A	-0.0182	0.0043	3.02E-05	1.36E-02
Ssc.9109.1.A1_at	ASGA0065917	14	116,719,950	C	A	-0.0191	0.0044	1.76E-05	1.26E-02

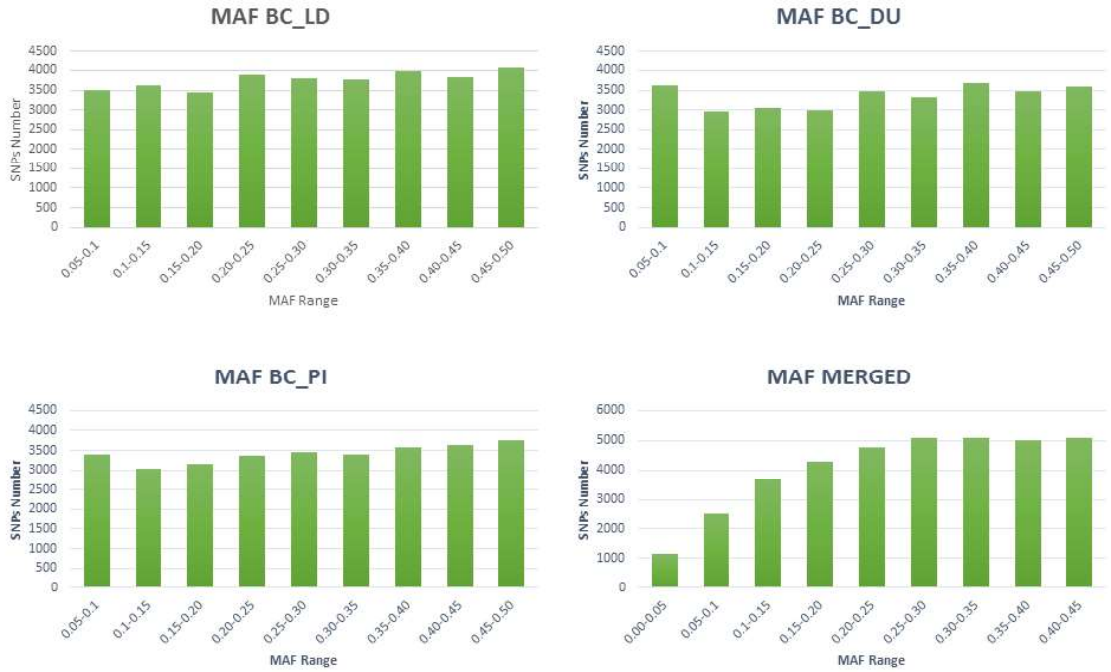
Ssc.9109.1.A1_at	ASGA0065921	14	116,740,764	G	A	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	ALGA0080825	14	116,759,572	A	G	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	ALGA0080826	14	116,773,141	A	G	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	H3GA0041906	14	116,796,765	A	G	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	DIAS0001032	14	116,827,002	A	G	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	H3GA0041907	14	116,855,028	A	G	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	DRGA0014462	14	117,091,767	A	G	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	DRGA0014464	14	117,140,679	G	A	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	MARC0026910	14	117,218,440	A	G	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	DRGA0014465	14	117,231,943	G	A	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	ASGA0065935	14	117,281,061	A	C	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	ALGA0080844	14	117,360,297	G	A	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	ALGA0080855	14	117,406,540	A	G	-0.0183	0.0047	1.30E-04	1.96E-02
Ssc.9109.1.A1_at	ASGA0065950	14	117,428,731	A	G	-0.0183	0.0047	1.30E-04	1.96E-02
Ssc.9109.1.A1_at	H3GA0041918	14	117,485,251	G	A	-0.0183	0.0047	1.30E-04	1.96E-02
Ssc.9109.1.A1_at	ASGA0065957	14	117,506,320	A	G	-0.0183	0.0047	1.30E-04	1.96E-02
Ssc.9109.1.A1_at	ALGA0080891	14	117,715,367	G	A	-0.0183	0.0047	1.30E-04	1.96E-02
Ssc.9109.1.A1_at	ALGA0080900	14	117,821,974	G	A	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	H3GA0041936	14	117,846,180	A	G	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	H3GA0041938	14	117,886,869	A	G	-0.0191	0.0044	1.76E-05	1.26E-02
Ssc.9109.1.A1_at	ASGA0065976	14	118,017,826	G	A	-0.0183	0.0047	1.30E-04	1.96E-02
Ssc.9109.1.A1_at	DIAS0000840	14	118,040,644	G	A	0.0182	0.0043	3.02E-05	1.36E-02
Ssc.9109.1.A1_at	ALGA0080908	14	118,072,947	G	A	0.0178	0.0045	1.06E-04	1.65E-02
Ssc.9109.1.A1_at	DIAS0002596	14	118,345,379	A	G	-0.0188	0.0051	2.70E-04	3.70E-02
Ssc.9109.1.A1_at	ALGA0080935	14	118,460,403	G	A	-0.0188	0.0051	2.70E-04	3.70E-02
Ssc.9109.1.A1_at	ASGA0066004	14	118,515,539	G	A	-0.0188	0.0051	2.70E-04	3.70E-02
Ssc.9109.1.A1_at	ALGA0080940	14	118,552,421	G	A	-0.0188	0.0051	2.70E-04	3.70E-02
Ssc.9109.1.A1_at	ALGA0080944	14	118,575,382	G	A	-0.0188	0.0051	2.70E-04	3.70E-02
Ssc.9109.1.A1_at	ALGA0080999	14	119,028,054	G	A	-0.0181	0.0049	2.50E-04	3.52E-02
Ssc.9109.1.A1_at	MARC0014536	14	120,439,354	A	G	-0.0184	0.0049	2.30E-04	3.26E-02
Ssc.30987.1.S1_at	DIAS0001428	14	135,271,348	C	A	-0.0702	0.0162	1.06E-07	8.35E-04
Ssc.30987.1.S1_at	ALGA0081794	14	135,296,757	G	A	-0.0698	0.0163	1.36E-07	8.61E-04
Ssc.30987.1.S1_at	DRGA0014615	14	135,333,817	G	A	-0.0590	0.0153	1.93E-06	6.11E-03
Ssc.30987.1.S1_at	ALGA0081801	14	135,352,519	A	G	-0.0590	0.0153	1.93E-06	6.11E-03
Ssc.30987.1.S1_at	ALGA0081804	14	135,366,386	G	A	-0.0590	0.0153	1.93E-06	6.11E-03
Ssc.30987.1.S1_at	ALGA0081834	14	135,671,638	G	A	0.0625	0.0169	5.23E-06	1.50E-02
Ssc.17853.1.A1_at	ALGA0081883	14	135,949,593	G	A	0.2781	0.0552	1.27E-07	3.85E-03
Ssc.30987.1.S1_at	ALGA0081883	14	135,949,593	G	A	-0.1331	0.0342	1.71E-06	6.11E-03
Ssc.30987.1.S1_at	ALGA0081886	14	135,991,043	A	G	-0.0746	0.0169	5.27E-08	7.15E-04
Ssc.30987.1.S1_at	DRGA0014640	14	136,239,246	A	C	0.0659	0.0168	1.34E-06	6.11E-03
Ssc.30987.1.S1_at	ASGA0066873	14	138,328,275	A	G	0.0713	0.0154	1.15E-08	3.63E-04
Ssc.3249.1.S1_at	MARC0018837	15	12,377,279	C	A	0.4075	0.0791	1.16E-06	1.18E-02
Ssc.3249.1.S1_at	ALGA0083911	15	14,225,608	A	G	0.3513	0.0752	1.03E-05	2.64E-02
Ssc.3249.1.S1_at	ASGA0068634	15	14,270,957	A	G	0.3513	0.0752	1.03E-05	2.64E-02
Ssc.3249.1.S1_at	H3GA0043770	15	14,321,621	A	G	0.3513	0.0752	1.03E-05	2.64E-02
Ssc.3249.1.S1_at	ALGA0120106	15	14,459,388	G	A	0.3513	0.0752	1.03E-05	2.64E-02
Ssc.3249.1.S1_at	ALGA0113095	15	14,514,100	C	A	0.3513	0.0752	1.03E-05	2.64E-02
Ssc.3249.1.S1_at	ALGA0083918	15	14,588,005	A	G	0.4075	0.0791	1.16E-06	1.18E-02
Ssc.3249.1.S1_at	DRGA0014948	15	14,889,528	A	G	0.4075	0.0791	1.16E-06	1.18E-02
Ssc.3249.1.S1_at	ASGA0068640	15	14,909,574	G	A	0.3513	0.0752	1.03E-05	2.64E-02
Ssc.3249.1.S1_at	ALGA0083923	15	14,940,640	G	A	0.3513	0.0752	1.03E-05	2.64E-02
Ssc.3249.1.S1_at	ALGA0083938	15	15,205,947	G	A	0.3594	0.0766	9.42E-06	2.64E-02
Ssc.3249.1.S1_at	ALGA0083951	15	15,286,888	A	G	0.3513	0.0752	1.03E-05	2.64E-02
Ssc.16050.1.A1_at	ALGA0086279	15	93,955,844	A	G	0.0853	0.0190	9.34E-06	1.92E-02
Ssc.18795.1.A1_at	ALGA0086279	15	93,955,844	A	G	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.16050.1.A1_at	ALGA0086365	15	99,540,054	A	C	0.0853	0.0190	9.34E-06	1.92E-02
Ssc.18795.1.A1_at	ALGA0086365	15	99,540,054	A	C	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.16050.1.A1_at	ALGA0086370	15	100,476,552	A	G	0.0853	0.0190	9.34E-06	1.92E-02

Ssc.18795.1.A1_at	ALGA0086370	15	100,476,552	A	G	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.16050.1.A1_at	INRA0049870	15	100,499,815	G	A	0.0853	0.0190	9.34E-06	1.92E-02
Ssc.18795.1.A1_at	INRA0049870	15	100,499,815	G	A	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.16050.1.A1_at	ALGA0086372	15	100,549,307	A	G	0.0853	0.0190	9.34E-06	1.92E-02
Ssc.18795.1.A1_at	ALGA0086372	15	100,549,307	A	G	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.16050.1.A1_at	ALGA0086374	15	100,583,138	G	A	0.0853	0.0190	9.34E-06	1.92E-02
Ssc.18795.1.A1_at	ALGA0086374	15	100,583,138	G	A	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.16050.1.A1_at	ALGA0086375	15	100,600,751	A	G	0.0853	0.0190	9.34E-06	1.92E-02
Ssc.18795.1.A1_at	ALGA0086375	15	100,600,751	A	G	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.16050.1.A1_at	ALGA0086376	15	100,618,897	G	A	0.0853	0.0190	9.34E-06	1.92E-02
Ssc.18795.1.A1_at	ALGA0086376	15	100,618,897	G	A	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.16050.1.A1_at	DRGA0015292	15	100,681,987	G	A	0.0853	0.0190	9.34E-06	1.92E-02
Ssc.18795.1.A1_at	DRGA0015292	15	100,681,987	G	A	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.16050.1.A1_at	ALGA0086378	15	101,125,967	C	A	0.0853	0.0190	9.34E-06	1.92E-02
Ssc.18795.1.A1_at	ALGA0086378	15	101,125,967	C	A	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.16050.1.A1_at	H3GA0044718	15	101,357,585	A	G	0.0853	0.0190	9.34E-06	1.92E-02
Ssc.18795.1.A1_at	H3GA0044718	15	101,357,585	A	G	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.16050.1.A1_at	ALGA0086391	15	101,437,317	G	A	0.0853	0.0190	9.34E-06	1.92E-02
Ssc.18795.1.A1_at	ALGA0086391	15	101,437,317	G	A	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.16050.1.A1_at	ALGA0086392	15	101,485,928	G	A	0.0853	0.0190	9.34E-06	1.92E-02
Ssc.18795.1.A1_at	ALGA0086392	15	101,485,928	G	A	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.16050.1.A1_at	MARC0042652	15	101,514,834	G	A	0.0853	0.0190	9.34E-06	1.92E-02
Ssc.18795.1.A1_at	MARC0042652	15	101,514,834	G	A	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.16050.1.A1_at	ASGA0070162	15	101,622,603	G	A	0.0853	0.0190	9.34E-06	1.92E-02
Ssc.18795.1.A1_at	ASGA0070162	15	101,622,603	G	A	0.0839	0.0209	6.35E-05	4.18E-02
Ssc.25378.1.S1_at	ASGA0071761	15	151,885,375	G	A	0.0990	0.0206	2.35E-07	6.94E-03
Ssc.7666.1.A1_at	ALGA0093386	17	14,849,296	A	G	0.1994	0.0445	2.07E-05	2.34E-02
Ssc.7666.1.A1_at	ALGA0105626	17	18,894,036	G	A	-0.1879	0.0415	1.68E-05	2.05E-02
Ssc.7666.1.A1_at	H3GA0047994	17	19,978,814	G	A	-0.1896	0.0441	4.39E-05	3.23E-02
Ssc.7666.1.A1_at	ALGA0093570	17	20,410,396	G	A	-0.1896	0.0441	4.39E-05	3.23E-02
Ssc.7666.1.A1_at	H3GA0048002	17	20,510,117	A	G	0.2063	0.0435	6.52E-06	1.30E-02
Ssc.7666.1.A1_at	M1GA0021675	17	20,631,316	G	A	0.1951	0.0413	6.89E-06	1.30E-02
Ssc.7666.1.A1_at	ALGA0093608	17	20,845,265	A	C	0.2072	0.0440	7.30E-06	1.30E-02
Ssc.7666.1.A1_at	ALGA0093636	17	21,320,339	A	G	0.1831	0.0429	4.85E-05	3.41E-02
Ssc.21242.1.S1_at	DRGA0016627	17	23,265,918	G	A	0.1529	0.0372	2.81E-05	4.23E-02
Ssc.7666.1.A1_at	DRGA0016627	17	23,265,918	G	A	0.1801	0.0392	1.21E-05	1.62E-02
Ssc.7666.1.A1_at	ALGA0093713	17	23,677,109	G	A	0.1854	0.0430	4.13E-05	3.23E-02
Ssc.7666.1.A1_at	ASGA0075727	17	23,699,267	G	A	0.1854	0.0430	4.13E-05	3.23E-02
Ssc.7666.1.A1_at	ASGA0075740	17	23,888,831	A	G	0.1854	0.0430	4.13E-05	3.23E-02
Ssc.7666.1.A1_at	H3GA0048100	17	25,327,008	A	G	0.1934	0.0415	9.07E-06	1.30E-02
Ssc.7666.1.A1_at	ASGA0075795	17	25,579,093	G	A	0.1934	0.0415	9.07E-06	1.30E-02
Ssc.7666.1.A1_at	ALGA0093822	17	25,614,746	C	A	0.1934	0.0415	9.07E-06	1.30E-02
Ssc.7666.1.A1_at	DBMA0000204	17	25,670,863	A	C	0.1934	0.0415	9.07E-06	1.30E-02
Ssc.21242.1.S1_at	MARC0091571	17	27,274,308	C	A	0.1784	0.0405	7.22E-06	1.81E-02
Ssc.21242.1.S1_at	ALGA0093942	17	28,475,777	A	G	-0.1485	0.0359	2.52E-05	4.23E-02
Ssc.7666.1.A1_at	ALGA0093942	17	28,475,777	A	G	-0.1737	0.0378	1.23E-05	1.62E-02
Ssc.21242.1.S1_at	ALGA0094007	17	29,684,825	G	A	0.1493	0.0373	4.42E-05	4.65E-02
Ssc.7666.1.A1_at	ALGA0094007	17	29,684,825	G	A	0.1999	0.0392	1.22E-06	1.08E-02
Ssc.7666.1.A1_at	H3GA0048371	17	33,892,898	G	A	0.1873	0.0397	7.39E-06	1.30E-02
Ssc.7666.1.A1_at	H3GA0054322	17	33,907,715	G	A	0.1782	0.0406	2.93E-05	3.09E-02
Ssc.7666.1.A1_at	ASGA0076304	17	34,888,048	G	A	0.1634	0.0392	7.19E-05	4.73E-02
Ssc.7666.1.A1_at	M1GA0021930	17	38,047,955	A	C	-0.1801	0.0385	8.63E-06	1.30E-02
Ssc.7666.1.A1_at	MARC0074465	17	38,382,031	A	G	-0.1801	0.0385	8.63E-06	1.30E-02
Ssc.7666.1.A1_at	DBNP0000274	17	38,521,013	A	G	-0.1644	0.0381	4.11E-05	3.23E-02
Ssc.7666.1.A1_at	ALGA0094640	17	38,688,258	A	C	-0.1801	0.0385	8.63E-06	1.30E-02
Ssc.7666.1.A1_at	H3GA0048717	17	38,816,919	A	G	-0.1801	0.0385	8.63E-06	1.30E-02
Ssc.21242.1.S1_at	ALGA0094743	17	40,556,315	A	G	-0.1664	0.0354	1.58E-06	8.31E-03
Ssc.7666.1.A1_at	ALGA0094743	17	40,556,315	A	G	-0.1814	0.0372	3.46E-06	1.30E-02

Ssc.21242.1.S1_at	ALGA0121030	17	41,558,311	C	A	0.1698	0.0360	1.52E-06	8.31E-03
Ssc.7666.1.A1_at	ALGA0121030	17	41,558,311	C	A	0.1628	0.0379	4.31E-05	3.23E-02
Ssc.21242.1.S1_at	MARC0020934	17	45,164,920	G	A	-0.1766	0.0411	1.16E-05	2.37E-02
Ssc.21242.1.S1_at	ALGA0095036	17	45,742,748	A	G	0.1665	0.0412	3.75E-05	4.65E-02
Ssc.21242.1.S1_at	ALGA0095111	17	46,491,832	A	G	0.1643	0.0412	4.73E-05	4.82E-02
Ssc.21242.1.S1_at	ALGA0095137	17	46,692,167	A	G	-0.1761	0.0387	3.45E-06	1.36E-02
Ssc.21242.1.S1_at	ASGA0077038	17	47,093,909	A	G	-0.1638	0.0375	8.46E-06	1.91E-02
Ssc.21242.1.S1_at	ASGA0077055	17	47,280,841	C	A	-0.1552	0.0387	4.24E-05	4.65E-02
Ssc.7666.1.A1_at	MARC0055696	17	47,981,889	G	A	0.1698	0.0378	1.94E-05	2.27E-02
Ssc.7666.1.A1_at	ALGA0095241	17	48,503,856	A	G	0.1887	0.0381	2.45E-06	1.30E-02
Ssc.21242.1.S1_at	MARC0015777	17	48,726,613	A	G	0.1466	0.0362	3.70E-05	4.65E-02
Ssc.7666.1.A1_at	MARC0015777	17	48,726,613	A	G	0.1653	0.0381	3.67E-05	3.23E-02
Ssc.7666.1.A1_at	ASGA0077119	17	48,924,365	G	A	-0.1919	0.0441	3.46E-05	3.23E-02
Ssc.21242.1.S1_at	MARC0009678	17	49,842,888	A	G	0.1833	0.0386	1.26E-06	8.31E-03
Ssc.7666.1.A1_at	MARC0009678	17	49,842,888	A	G	0.2131	0.0406	5.81E-07	1.08E-02
Ssc.21242.1.S1_at	ALGA0115372	17	49,910,115	A	G	0.1550	0.0377	2.72E-05	4.23E-02
Ssc.7666.1.A1_at	ALGA0115372	17	49,910,115	A	G	0.1690	0.0396	4.96E-05	3.41E-02
Ssc.21242.1.S1_at	ALGA0095309	17	50,043,867	A	C	0.1630	0.0371	7.45E-06	1.81E-02
Ssc.21242.1.S1_at	MARC0039767	17	50,152,171	A	G	0.1630	0.0371	7.45E-06	1.81E-02
Ssc.21242.1.S1_at	ASGA0077203	17	51,638,480	G	A	0.1919	0.0404	1.25E-06	8.31E-03
Ssc.7666.1.A1_at	ASGA0077203	17	51,638,480	G	A	0.2172	0.0425	1.14E-06	1.08E-02
Ssc.21242.1.S1_at	SIRI0000886	17	52,124,246	G	A	0.1755	0.0377	2.02E-06	9.10E-03
Ssc.7666.1.A1_at	SIRI0000886	17	52,124,246	G	A	0.1862	0.0396	7.79E-06	1.30E-02
Ssc.21242.1.S1_at	ALGA0095422	17	52,150,042	A	G	0.1783	0.0370	9.20E-07	8.31E-03
Ssc.7666.1.A1_at	ALGA0095422	17	52,150,042	A	G	0.1823	0.0390	8.51E-06	1.30E-02
Ssc.21242.1.S1_at	ASGA0077273	17	52,815,840	A	C	-0.1783	0.0398	5.03E-06	1.61E-02
Ssc.7666.1.A1_at	ALGA0095523	17	54,025,507	G	A	-0.1862	0.0398	8.34E-06	1.30E-02
Ssc.7666.1.A1_at	ALGA0095529	17	54,053,115	C	A	-0.1787	0.0382	8.47E-06	1.30E-02
Ssc.7666.1.A1_at	ASGA0077354	17	54,136,444	G	A	-0.2163	0.0494	3.13E-05	3.20E-02
Ssc.7666.1.A1_at	H3GA0049266	17	54,147,838	A	G	-0.1886	0.0443	5.20E-05	3.50E-02
Ssc.21242.1.S1_at	ASGA0091871	17	54,774,793	A	G	-0.1885	0.0459	2.80E-05	4.23E-02
Ssc.7666.1.A1_at	MARC0114143	17	56,096,189	G	A	0.1655	0.0385	4.29E-05	3.23E-02
Ssc.21242.1.S1_at	INRA0054308	17	58,236,219	C	A	0.1747	0.0410	1.39E-05	2.58E-02
Ssc.24997.1.S1_at	ALGA0096651	17	68,079,954	A	C	0.0822	0.0199	2.27E-06	1.25E-02

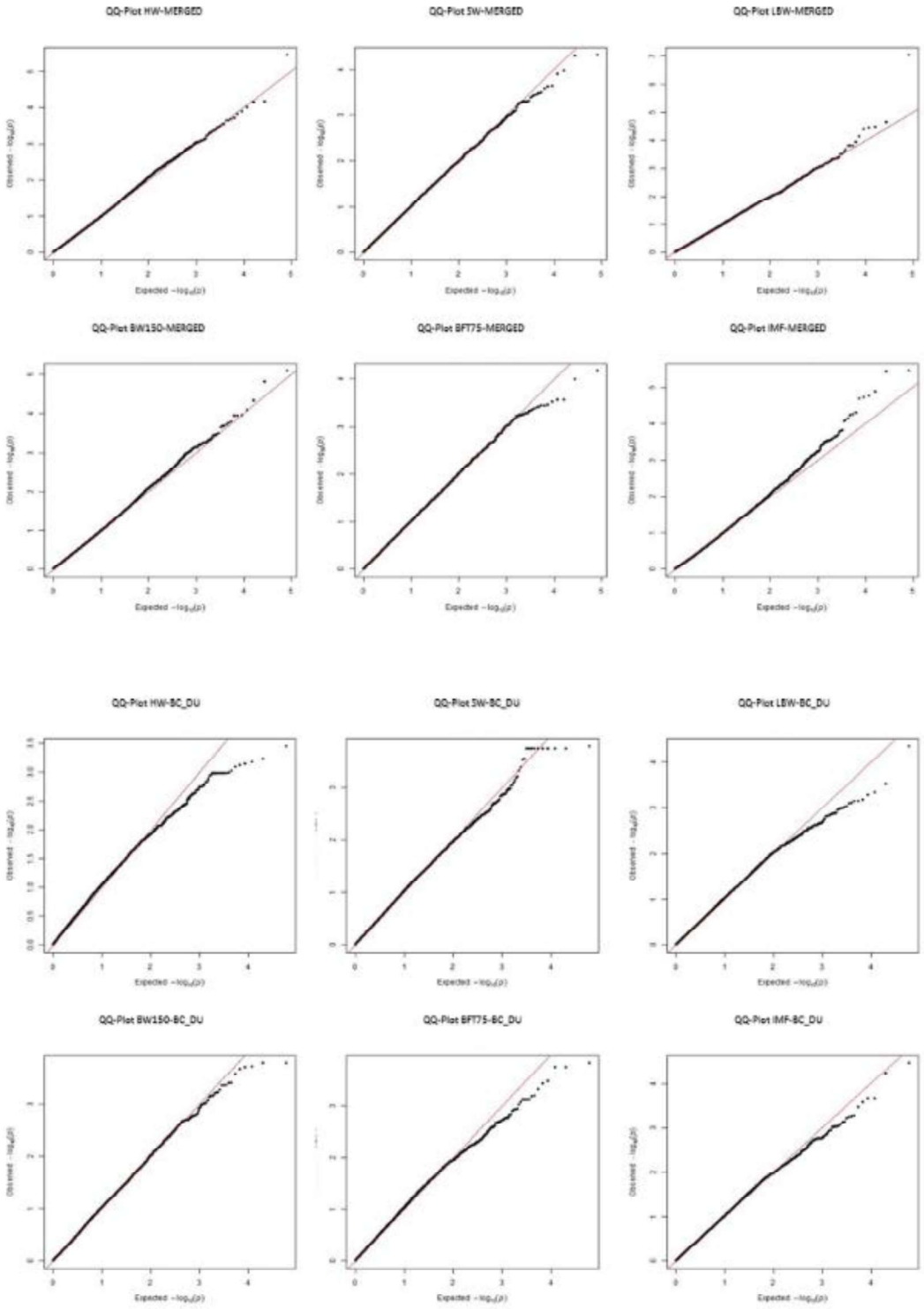
ARTÍCULO 3: Using genome wide association studies to identify common and specific QTL in three different genetic backgrounds based on Iberian pig breed.

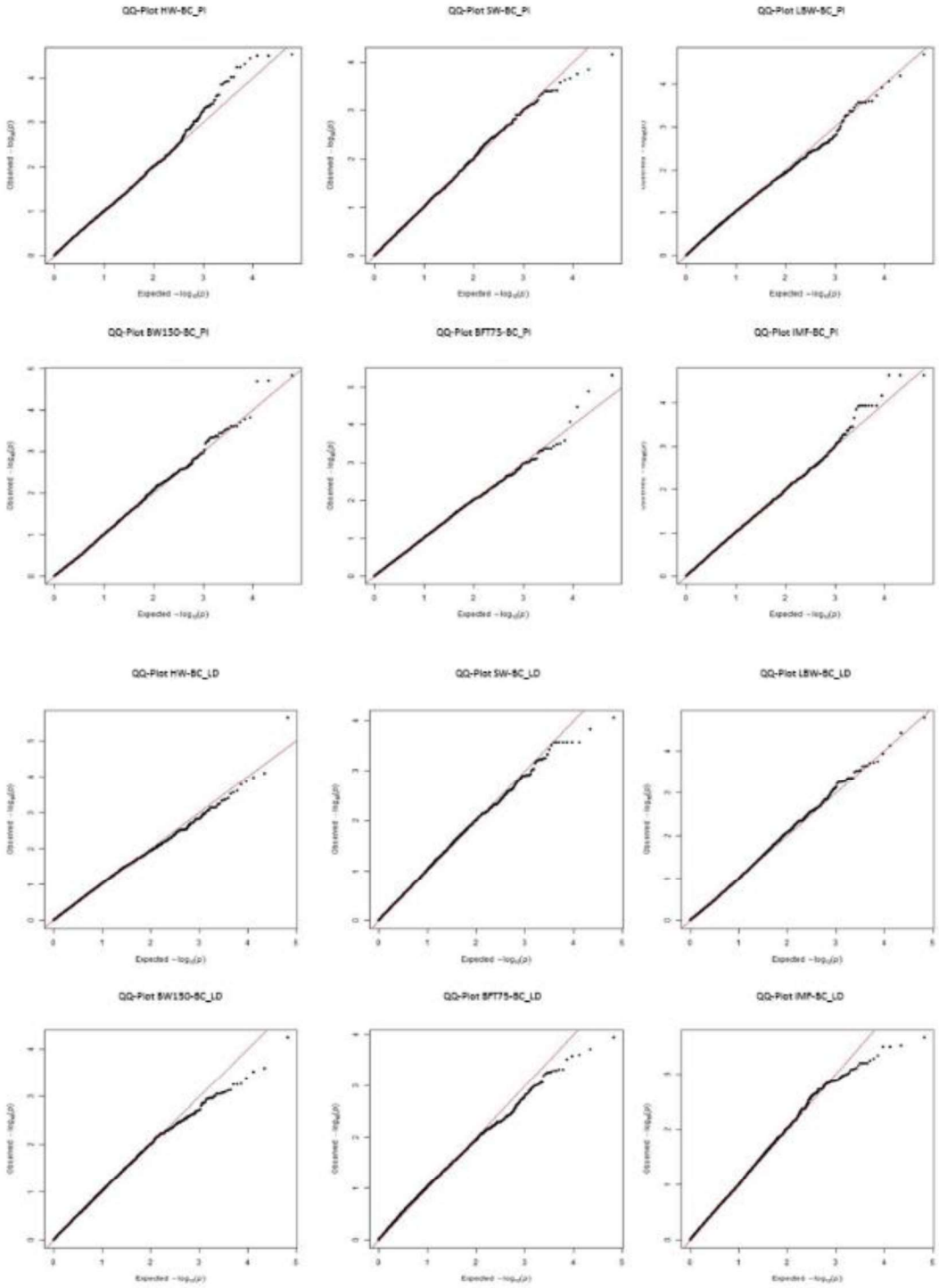
S1 Figure: Minimum allele frequency (MAF) values of the SNPs included in each of the datasets analyzed (BC_LD, BC_DI, BC_DU and Merged dataset).



ARTÍCULO 3: Using genome wide association studies to identify common and specific QTL in three different genetic backgrounds based on Iberian pig breed.

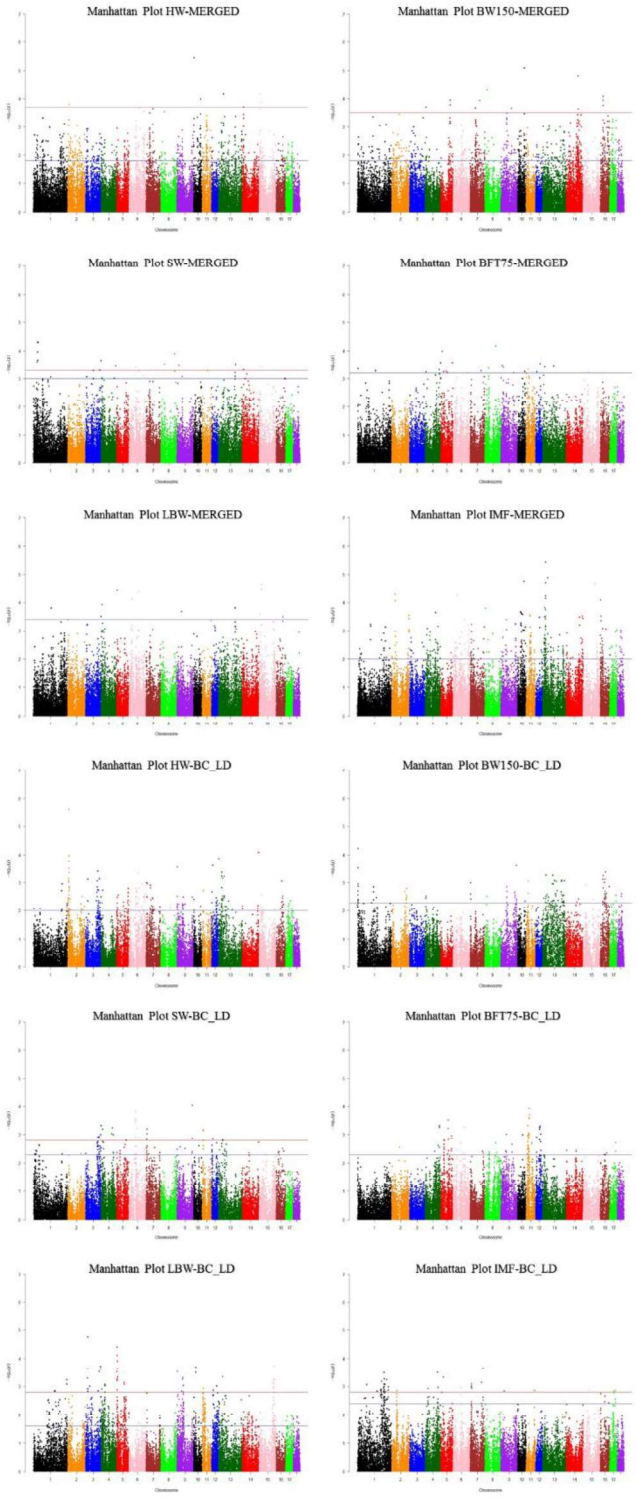
S2 Figure: QQ-Plots for each of the GWAS analysis carried out with each of the datasets (BC_LD, BC_DI, BC_DU and Merged dataset) and the six phenotypic traits (BW150, BFT75, HW, SW, LBW and IMF)

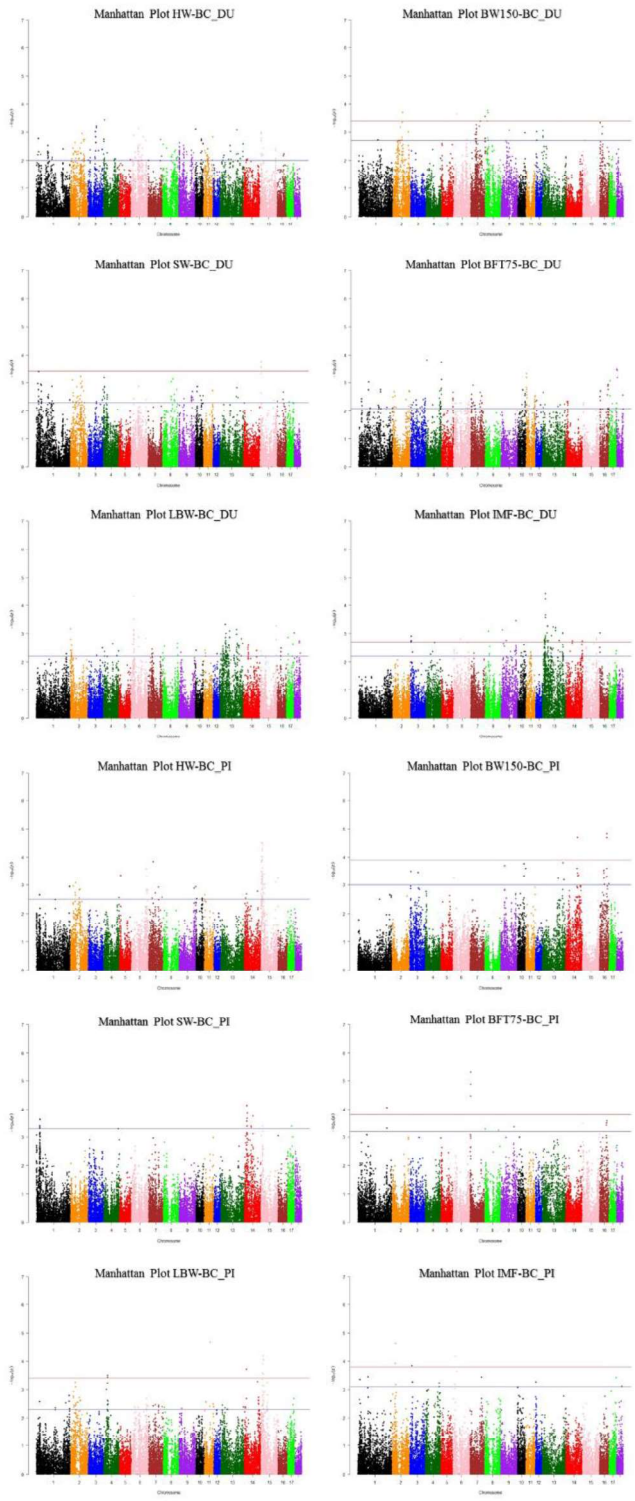



QQ-Plot HW-BC_PI
QQ-Plot SW-BC_PI
QQ-Plot LBW-BC_PI
QQ-Plot BW150-BC_PI
QQ-Plot BFT75-BC_PI
QQ-Plot IMF-BC_PI
QQ-Plot HW-BC_LD
QQ-Plot SW-BC_LD
QQ-Plot LBW-BC_LD
QQ-Plot BW150-BC_LD
QQ-Plot BFT75-BC_LD
QQ-Plot IMF-BC_LD

ARTÍCULO 3: Using genome wide association studies to identify common and specific QTL in three different genetic backgrounds based on Iberian pig breed.

S3 Figure: Manhattan Plots for each one of the GWAS analysis carried out with each dataset (BC_LD, BC_DI, BC_DU and Merged dataset) and the phenotypic traits BW150, BFT75, HW, SW, LBW and IMF. Significance thresholds were estimated using QQ-Plot approach.




Manhattan Plot HW-BC_DU
Manhattan Plot BW150-BC_DU
Manhattan Plot SW-BC_DU
Manhattan Plot BFT75-BC_DU
Manhattan Plot LBW-BC_DU
Manhattan Plot IMF-BC_DU
Manhattan Plot HW-BC_PI
Manhattan Plot BW150-BC_PI
Manhattan Plot SW-BC_PI
Manhattan Plot BFT75-BC_PI
Manhattan Plot LBW-BC_PI
Manhattan Plot IMF-BC_PI

ARTÍCULO 3: Using genome wide association studies to identify common and specific QTL in three different genetic backgrounds based on Iberian pig breed.

S4 Table: Candidate SNPs identified for the candidate genes selected in the common regions, backcross-specific regions and merged dataset regions. Genomic location, reference allele (Ref) and alternative allele (Alt).

COMMON REGIONS					
SNP Name	Gene	Chr	Position	Ref	Alt
COMMON					
EGFLAM_16_24894486	EGFLAM	16	24,894,486	C	A
EGFLAM_16_24788377	EGFLAM	16	24,788,377	T	C
EGFLAM_16_24828786	EGFLAM	16	24,828,786	C	T
EGFLAM_16_24831964	EGFLAM	16	24,831,964	C	A
CAST_2_107105096	CAST	2	107,105,096	G	C
CAST_2_107105104	CAST	2	107,105,104	G	A
CAST_2_107105154	CAST	2	107,105,154	T	C
CAST_2_107114719	CAST	2	107,114,719	T	C
ERAP1_2_107142877	ERAP1	2	107,142,877	A	G
ERAP1_2_107144675	ERAP1	2	107,144,675	C	T
ERAP1_2_107151819	ERAP1	2	107,151,819	T	G
ERAP1_2_107151849	ERAP1	2	107,151,849	T	G
ERAP1_2_107151925	ERAP1	2	107,151,925	C	T
ERAP1_2_107152758	ERAP1	2	107,152,758	A	G
ERAP1_2_107152782	ERAP1	2	107,152,782	G	A
ERAP1_2_107155636	ERAP1	2	107,155,636	G	T
ERAP1_2_107155738	ERAP1	2	107,155,738	A	G
ERAP1_2_107155747	ERAP1	2	107,155,747	A	C
ERAP1_2_107159001	ERAP1	2	107,159,001	A	G
PAM_2_112535654	PAM	2	112,535,654	C	T
PAM_2_112548977	PAM	2	112,548,977	A	C
PAM_2_112553825	PAM	2	112,553,825	A	T
IL4R_3_19848207	IL4R	3	19,848,207	G	A
IL4R_3_19848238	IL4R	3	19,848,238	A	T
IL4R_3_19848824	IL4R	3	19,848,824	C	T
MLH1_13_23643193	MLH1	13	23,643,193	A	G
MLH1_13_23643205	MLH1	13	23,643,205	A	C
MLH1_13_23643257	MLH1	13	23,643,257	A	T
MLH1_13_23643274	MLH1	13	23,643,274	C	T
MLH1_13_23643287	MLH1	13	23,643,287	A	G
MLH1_13_23643301	MLH1	13	23,643,301	G	A
MLH1_13_23653771	MLH1	13	23,653,771	A	G
MLH1_13_23653815	MLH1	13	23,653,815	C	G
MLH1_13_23653910	MLH1	13	23,653,910	A	G
MLH1_13_23690388	MLH1	13	23,690,388	T	C

MERGE					
CAV1_18_31746137	CAV1	18	31,746,137	A	G
CAV1_18_31747600	CAV1	18	31,747,600	C	T
CAV1_18_31747687	CAV1	18	31,747,687	G	C
CAV1_18_31778902	CAV1	18	31,778,902	G	A
CAV1_18_31746065	CAV1	18	31,746,065	G	A

BC_DU					
SNP Name	Gene	Chr	Position	Ref	Alt
MUSK_1_282500018	MUSK	1	282,500,018	A	G
MUSK_1_282504509	MUSK	1	282,504,509	T	C
ASS1_1_304475278	ASS1	1	304,475,278	G	C
ASS1_1_304475529	ASS1	1	304,475,529	G	A
ASS1_1_304486983	ASS1	1	304,486,983	C	T
ASS1_1_304487130	ASS1	1	304,487,130	C	T
ASS1_1_304487692	ASS1	1	304,487,692	A	C
ASS1_1_304488172	ASS1	1	304,488,172	G	T
ASS1_1_304488747	ASS1	1	304,488,747	C	T
ASS1_1_304488748	ASS1	1	304,488,748	A	T
ASS1_1_304490387	ASS1	1	304,490,387	C	T
SUCLA2_11_20078913	SUCLA2	11	20,078,913	A	G
SUCLA2_11_20118625	SUCLA2	11	20,118,625	A	G
SUCLA2_11_20122477	SUCLA2	11	20,122,477	A	G
KRT8_5_18666771	KRT8	5	18,666,771	G	A
KRT8_5_18666770	KRT8	5	18,666,770	C	T
KRT8_5_18666673	KRT8	5	18,666,673	T	C
KRT8_5_18666671	KRT8	5	18,666,671	T	C
KRT8_5_18666655	KRT8	5	18,666,655	A	G
KRT8_5_18666107	KRT8	5	18,666,107	C	T
KRT8_5_18665147	KRT8	5	18,665,147	G	T
KRT8_5_18664763	KRT8	5	18,664,763	A	G
DSP_7_5020813	DSP	7	5,020,813	C	T

BC_PI					
SNP Name	Gene	Chr	Position	Ref	Alt
PRKDC_4_87252744	PRKDC	4	87,252,744	C	T
PRKDC_4_87305239	PRKDC	4	87,305,239	C	T
PRKDC_4_87306994	PRKDC	4	87,306,994	G	A
PRKDC_4_87310345	PRKDC	4	87,310,345	G	T
SELL_4_88931558	SELL	4	88,931,558	G	A
SELL_4_88950415	SELL	4	88,950,415	T	C
SELL_4_88950439	SELL	4	88,950,439	A	G
SELP_4_89025668	SELP	4	89,025,668	T	C
KARS_6_11903140	KARS	6	11,903,140	A	G
KARS_6_11907863	KARS	6	11,907,863	G	A

KARS_6_11908169	KARS	6	11,908,169	G	A
KARS_6_11910088	KARS	6	11,910,088	C	T
KARS_6_11911405	KARS	6	11,911,405	A	G
KARS_6_11911429	KARS	6	11,911,429	C	T
KARS_6_11911980	KARS	6	11,911,980	C	T
KARS_6_11912112	KARS	6	11,912,112	G	A
KARS_6_11914497	KARS	6	11,914,497	G	A
KARS_6_11914573	KARS	6	11,914,573	C	T
HP_6_14644483	HP	6	14,644,483	T	G
HP_6_14644494	HP	6	14,644,494	G	C
HP_6_14644715	HP	6	14,644,715	G	A
HP_6_14646065	HP	6	14,646,065	G	A
CXCR4_15_18127836	CXCR4	15	18,127,836	C	G

S5 Table: Effect prediction for candidate SNPs identified on candidate genes, using VeP tool (Edited Table)

Uploaded variant	Symbol	Location	Allele	Consequence	Impact	Exon	Intron
MUSK_1_282500018	MUSK	1:282500018-282500018	G	intron_variant	MODIFIER	-	11//26
MUSK_1_282500018	MUSK	1:282500018-282500018	G	intron_variant	MODIFIER	-	11//27
MUSK_1_282504509	MUSK	1:282504509-282504509	C	intron_variant	MODIFIER	-	12//26
MUSK_1_282504509	MUSK	1:282504509-282504509	C	intron_variant	MODIFIER	-	12//27
ASS1_1_304475278	ASS1	1:304475278-304475278	C	intron_variant	MODIFIER	-	6//14
ASS1_1_304475529	ASS1	1:304475529-304475529	A	intron_variant	MODIFIER	-	6//14
ASS1_1_304486983	ASS1	1:304486983-304486983	T	intron_variant	MODIFIER	-	10//14
ASS1_1_304487130	ASS1	1:304487130-304487130	T	intron_variant	MODIFIER	-	11//14
ASS1_1_304487692	ASS1	1:304487692-304487692	C	intron_variant	MODIFIER	-	11//14
ASS1_1_304488172	ASS1	1:304488172-304488172	T	intron_variant	MODIFIER	-	11//14
ASS1_1_304488747	ASS1	1:304488747-304488747	T	intron_variant	MODIFIER	-	11//14
ASS1_1_304488748	ASS1	1:304488748-304488748	T	intron_variant	MODIFIER	-	11//14
ASS1_1_304490387	ASS1	1:304490387-304490387	T	intron_variant	MODIFIER	-	11//14
CAST_2_107105096	CAST	2:107105096-107105096	C	intron_variant	MODIFIER	-	20//30
CAST_2_107105096	CAST	2:107105096-107105096	C	intron_variant	MODIFIER	-	19//29
CAST_2_107105104	CAST	2:107105104-107105104	A	intron_variant	MODIFIER	-	20//30
CAST_2_107105104	CAST	2:107105104-107105104	A	intron_variant	MODIFIER	-	19//29
CAST_2_107105154	CAST	2:107105154-107105154	C	intron_variant	MODIFIER	-	20//30
CAST_2_107105154	CAST	2:107105154-107105154	C	intron_variant	MODIFIER	-	19//29
CAST_2_107114719	CAST	2:107114719-107114719	C	synonymous_variant	LOW	27//31	-
CAST_2_107114719	CAST	2:107114719-107114719	C	synonymous_variant	LOW	26//30	-
ERAP1_2_107142877	ERAP1	2:107142877-107142877	G	synonymous_variant	LOW	12//21	-
ERAP1_2_107142877	ERAP1	2:107142877-107142877	G	synonymous_variant	LOW	11//16	-
ERAP1_2_107144675	ERAP1	2:107144675-107144675	T	synonymous_variant	LOW	11//21	-
ERAP1_2_107144675	ERAP1	2:107144675-107144675	T	synonymous_variant	LOW	10//16	-
ERAP1_2_107151819	ERAP1	2:107151819-107151819	G	missense_variant	MODERATE	6//21	-
ERAP1_2_107151819	ERAP1	2:107151819-107151819	G	missense_variant	MODERATE	5//16	-
ERAP1_2_107151849	ERAP1	2:107151849-107151849	G	missense_variant	MODERATE	6//21	-
ERAP1_2_107151849	ERAP1	2:107151849-107151849	G	missense_variant	MODERATE	5//16	-
ERAP1_2_107151925	ERAP1	2:107151925-107151925	T	missense_variant	MODERATE	6//21	-
ERAP1_2_107151925	ERAP1	2:107151925-107151925	T	missense_variant	MODERATE	5//16	-
ERAP1_2_107152758	ERAP1	2:107152758-107152758	G	synonymous_variant	LOW	5//21	-

ERAP1_2_107152758	ERAP1	2:107152758-107152758	G	synonymous_variant	LOW	4//16	-
ERAP1_2_107152782	ERAP1	2:107152782-107152782	A	synonymous_variant	LOW	5//21	-
ERAP1_2_107152782	ERAP1	2:107152782-107152782	A	synonymous_variant	LOW	4//16	-
ERAP1_2_107155636	ERAP1	2:107155636-107155636	T	synonymous_variant	LOW	4//21	-
ERAP1_2_107155636	ERAP1	2:107155636-107155636	T	synonymous_variant	LOW	3//16	-
ERAP1_2_107155738	ERAP1	2:107155738-107155738	G	synonymous_variant	LOW	4//21	-
ERAP1_2_107155738	ERAP1	2:107155738-107155738	G	synonymous_variant	LOW	3//16	-
ERAP1_2_107155747	ERAP1	2:107155747-107155747	C	synonymous_variant	LOW	4//21	-
ERAP1_2_107155747	ERAP1	2:107155747-107155747	C	synonymous_variant	LOW	3//16	-
ERAP1_2_107159001	ERAP1	2:107159001-107159001	G	missense_variant	MODERATE	3//21	-
ERAP1_2_107159001	ERAP1	2:107159001-107159001	G	missense_variant	MODERATE	2//16	-
PAM_2_112535654	PAM	2:112535654-112535654	T	missense_variant	MODERATE	19//25	-
PAM_2_112548977	PAM	2:112548977-112548977	C	missense_variant	MODERATE	23//25	-
PAM_2_112553825	PAM	2:112553825-112553825	T	3_prime_UTR_variant	MODIFIER	25//25	-
IL4R_3_19848207	IL4R	3:19848207-19848207	A	synonymous_variant	LOW	9//9	-
IL4R_3_19848207	IL4R	3:19848207-19848207	A	upstream_gene_variant	MODIFIER	-	-
IL4R_3_19848207	IL4R	3:19848207-19848207	A	downstream_gene_variant	MODIFIER	-	-
IL4R_3_19848238	IL4R	3:19848238-19848238	T	missense_variant	MODERATE	9//9	-
IL4R_3_19848238	IL4R	3:19848238-19848238	T	upstream_gene_variant	MODIFIER	-	-
IL4R_3_19848238	IL4R	3:19848238-19848238	T	downstream_gene_variant	MODIFIER	-	-
IL4R_3_19848824	IL4R	3:19848824-19848824	T	synonymous_variant	LOW	8//9	-
IL4R_3_19848824	IL4R	3:19848824-19848824	T	upstream_gene_variant	MODIFIER	-	-
IL4R_3_19848824	IL4R	3:19848824-19848824	T	synonymous_variant	LOW	8//8	-
PRKDC_4_87252744	PRKDC	4:87252744-87252744	T	synonymous_variant	LOW	31//88	-
PRKDC_4_87305239	PRKDC	4:87305239-87305239	T	synonymous_variant	LOW	66//88	-
PRKDC_4_87306994	PRKDC	4:87306994-87306994	A	synonymous_variant	LOW	68//88	-
PRKDC_4_87310345	PRKDC	4:87310345-87310345	T	synonymous_variant	LOW	70//88	-
SELL_4_88931558	SELL	4:88931558-88931558	A	5_prime_UTR_variant	MODIFIER	1//9	-
SELL_4_88931558	SELL	4:88931558-88931558	A	upstream_gene_variant	MODIFIER	-	-
SELL_4_88950415	SELL	4:88950415-88950415	C	3_prime_UTR_variant	MODIFIER	9//9	-
SELL_4_88950439	SELL	4:88950439-88950439	G	3_prime_UTR_variant	MODIFIER	9//9	-
SELP_4_89025668	SELP	4:89025668-89025668	C	3_prime_UTR_variant	MODIFIER	14//14	-
KRT8_5_18664763	KRT8	5:18664763-18664763	G	intron_variant	MODIFIER	-	6//8
KRT8_5_18665147	KRT8	5:18665147-18665147	T	intron_variant	MODIFIER	-	5//8
KRT8_5_18666107	KRT8	5:18666107-18666107	T	intron_variant	MODIFIER	-	4//8
KRT8_5_18666655	KRT8	5:18666655-18666655	G	intron_variant	MODIFIER	-	3//8
KRT8_5_18666671	KRT8	5:18666671-18666671	C	intron_variant	MODIFIER	-	3//8

KRT8_5_18666673	KRT8	5:18666673-18666673	C	intron_variant	MODIFIER	-	3//8
KRT8_5_18666770	KRT8	5:18666770-18666770	T	intron_variant	MODIFIER	-	3//8
KRT8_5_18666771	KRT8	5:18666771-18666771	A	intron_variant	MODIFIER	-	3//8
KARS_6_11903140	KARS	6:11903140-11903140	G	synonymous_variant	LOW	2//14	-
KARS_6_11907863	KARS	6:11907863-11907863	A	missense_variant	MODERATE	5//14	-
KARS_6_11908169	KARS	6:11908169-11908169	A	synonymous_variant	LOW	6//14	-
KARS_6_11910088	KARS	6:11910088-11910088	T	synonymous_variant	LOW	7//14	-
KARS_6_11911405	KARS	6:11911405-11911405	G	synonymous_variant	LOW	8//14	-
KARS_6_11911429	KARS	6:11911429-11911429	T	synonymous_variant	LOW	8//14	-
KARS_6_11911980	KARS	6:11911980-11911980	T	synonymous_variant	LOW	10//14	-
KARS_6_11912112	KARS	6:11912112-11912112	A	intron_variant	MODIFIER	-	10//13
KARS_6_11914497	KARS	6:11914497-11914497	A	3_prime_UTR_variant	MODIFIER	14//14	-
KARS_6_11914573	KARS	6:11914573-11914573	T	3_prime_UTR_variant	MODIFIER	14//14	-
HP_6_14644483	HP	6:14644483-14644483	G	intron_variant	MODIFIER	-	1//4
HP_6_14644483	HP	6:14644483-14644483	G	upstream_gene_variant	MODIFIER	-	-
HP_6_14644483	HP	6:14644483-14644483	G	intron_variant	MODIFIER	-	1//3
HP_6_14644483	HP	6:14644483-14644483	G	intron_variant	MODIFIER	-	1//5
HP_6_14644483	HP	6:14644483-14644483	G	intron_variant	MODIFIER	-	1//4
HP_6_14644483	HP	6:14644483-14644483	G	intron_variant	MODIFIER	-	1//4
HP_6_14644483	HP	6:14644483-14644483	G	upstream_gene_variant	MODIFIER	-	-
HP_6_14644494	HP	6:14644494-14644494	C	intron_variant	MODIFIER	-	1//4
HP_6_14644494	HP	6:14644494-14644494	C	upstream_gene_variant	MODIFIER	-	-
HP_6_14644494	HP	6:14644494-14644494	C	intron_variant	MODIFIER	-	1//3
HP_6_14644494	HP	6:14644494-14644494	C	intron_variant	MODIFIER	-	1//5
HP_6_14644494	HP	6:14644494-14644494	C	intron_variant	MODIFIER	-	1//4
HP_6_14644494	HP	6:14644494-14644494	C	intron_variant	MODIFIER	-	1//4
HP_6_14644494	HP	6:14644494-14644494	C	upstream_gene_variant	MODIFIER	-	-
HP_6_14644715	HP	6:14644715-14644715	A	intron_variant	MODIFIER	-	1//4
HP_6_14644715	HP	6:14644715-14644715	A	upstream_gene_variant	MODIFIER	-	-
HP_6_14644715	HP	6:14644715-14644715	A	intron_variant	MODIFIER	-	1//3
HP_6_14644715	HP	6:14644715-14644715	A	5_prime_UTR_variant	MODIFIER	2//6	-
HP_6_14644715	HP	6:14644715-14644715	A	intron_variant	MODIFIER	-	1//4
HP_6_14644715	HP	6:14644715-14644715	A	intron_variant	MODIFIER	-	1//4
HP_6_14644715	HP	6:14644715-14644715	A	upstream_gene_variant	MODIFIER	-	-
HP_6_14646065	HP	6:14646065-14646065	A	intron_variant	MODIFIER	-	3//4
HP_6_14646065	HP	6:14646065-14646065	A	intron_variant, non_coding_transcript_variant	MODIFIER	-	2//3
HP_6_14646065	HP	6:14646065-14646065	A	intron_variant	MODIFIER	-	2//3

HP_6_14646065	HP	6:14646065-14646065	A	intron_variant	MODIFIER	-	4//5
HP_6_14646065	HP	6:14646065-14646065	A	intron_variant	MODIFIER	-	3//4
HP_6_14646065	HP	6:14646065-14646065	A	intron_variant	MODIFIER	-	3//4
HP_6_14646065	HP	6:14646065-14646065	A	downstream_gene_variant	MODIFIER	-	-
DSP_7_5020813	DSP	7:5020813-5020813	T	3_prime_UTR_variant	MODIFIER	26//26	-
SUCLA2_11_20078913	SUCLA2	11:20078913-20078913	G	intron_variant	MODIFIER	-	2//10
SUCLA2_11_20118625	SUCLA2	11:20118625-20118625	G	intron_variant	MODIFIER	-	10//10
SUCLA2_11_20122477	SUCLA2	11:20122477-20122477	G	intron_variant	MODIFIER	-	10//10
MLH1_13_23643193	MLH1	13:23643193-23643193	G	intron_variant	MODIFIER	-	10//21
MLH1_13_23643205	MLH1	13:23643205-23643205	C	intron_variant	MODIFIER	-	10//21
MLH1_13_23643257	MLH1	13:23643257-23643257	T	intron_variant	MODIFIER	-	10//21
MLH1_13_23643274	MLH1	13:23643274-23643274	T	intron_variant	MODIFIER	-	10//21
MLH1_13_23643287	MLH1	13:23643287-23643287	G	intron_variant	MODIFIER	-	10//21
MLH1_13_23643301	MLH1	13:23643301-23643301	A	intron_variant	MODIFIER	-	10//21
MLH1_13_23653771	MLH1	13:23653771-23653771	G	synonymous_variant	LOW	15//22	-
MLH1_13_23653815	MLH1	13:23653815-23653815	G	missense_variant	MODERATE	15//22	-
MLH1_13_23653910	MLH1	13:23653910-23653910	G	missense_variant	MODERATE	15//22	-
MLH1_13_23690388	MLH1	13:23690388-23690388	C	synonymous_variant	LOW	21//22	-
CXCR4_15_18127836	CXCR4	15:18127836-18127836	G	synonymous_variant	LOW	2//2	-
CXCR4_15_18127836	CXCR4	15:18127836-18127836	G	non_coding_transcript_exon_variant, non_coding_transcript_variant	MODIFIER	2//2	-
EGFLAM_16_24788377	EGFLAM	16:24788377-24788377	C	synonymous_variant	LOW	6//28	-
EGFLAM_16_24788377	EGFLAM	16:24788377-24788377	C	synonymous_variant	LOW	6//28	-
EGFLAM_16_24828786	EGFLAM	16:24828786-24828786	T	synonymous_variant	LOW	11//28	-
EGFLAM_16_24828786	EGFLAM	16:24828786-24828786	T	synonymous_variant	LOW	11//28	-
EGFLAM_16_24831964	EGFLAM	16:24831964-24831964	A	synonymous_variant	LOW	12//28	-
EGFLAM_16_24831964	EGFLAM	16:24831964-24831964	A	synonymous_variant	LOW	12//28	-
EGFLAM_16_24894486	EGFLAM	16:24894486-24894486	A	missense_variant	MODERATE	26//28	-
EGFLAM_16_24894486	EGFLAM	16:24894486-24894486	A	missense_variant	MODERATE	26//28	-
CAV1_18_31746065	CAV1	18:31746065-31746065	A	3_prime_UTR_variant	MODIFIER	3//3	-
CAV1_18_31746137	CAV1	18:31746137-31746137	G	3_prime_UTR_variant	MODIFIER	3//3	-
CAV1_18_31747600	CAV1	18:31747600-31747600	T	synonymous_variant	LOW	3//3	-
CAV1_18_31747687	CAV1	18:31747687-31747687	C	synonymous_variant	LOW	3//3	-
CAV1_18_31778902	CAV1	18:31778902-31778902	A	intron_variant	MODIFIER	-	1//2